THE BREWER'S ANALYST

R. DOUGLAS BAILEY

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A SYSTEMATIC HANDBOOK OF ANALYSIS RELATING
TO BREWING AND MALTING

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GIVING DETAILS OF UP-TO-DATE METHODS OF ANALYSING ALL MATERIALS USED, AND PRODUCTS MANUFACTURED, BY BREWERS AND MALTSTERS

TOGETHER WITH

INTERPRETATION OF ANALYSES, POLARISCOPICAL, MICROSCOPICAL, AND BIOLOGICAL WORK

BY

R. DOUGLAS BAILEY, F.C.S., F.R.M.S.

WITH NUMEROUS ILLUSTRATIONS

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PREFACE.

The author's first work, dealing amongst other matters with the analyses of brewing materials and products, was published sixteen years ago, entitled *Notes on Brewing*, being a collection of the more important of his articles contributed during several years to the *Brewers' Guardian*. Since that time it has been his occasional occupation to write articles on brewing for one of the trade journals and to carry out analytical work for several large brewing concerns. To this it may be added that for the past eighteen years he has been daily employed in conducting practical brewing and malting operations on an extensive scale, and can therefore, with reason, claim to have a practical and scientific knowledge of the subjects which his present work treats. So far as he is aware, there are but two works dealing solely with analyses relating to brewing—one having been published so far back as 1884, and slightly revised some six years ago, the other, more recent, being a drawn-up course of laboratory studies for the special use of the students at the Birmingham University. There can be hardly any doubt under these circumstances that there is at present a want for an up-to-date work for the use of brewers and brewing students, and it is to supply this want that the author has published the present volume.

Of late years there has been considerable controversy amongst brewers' analysts as to the standardisation of analytical methods; and although nothing definite has so far been decided, the author has borne the controversy in mind, and, in view thereof, has endeavoured to steer clear of the same, and not vary the generally employed methods of analysis more than is consistent with modern views, which have resulted in more accurate information in the evaluation of brewing materials being obtained.

Details are given of the polarisation of light, a subject neglected in all other works on brewing; particulars are also given of the latest improvements in the polarimeter. A chapter is

devoted to arsenical work, which is also of the greatest importance; whilst in the appendices is given a series of tables and typical analyses which, it is believed, will prove extremely useful, particularly to those about to commence a laboratory course.

The author has left the consideration of the biological examination of water, malt, hops, and beer out of the part dealing with methods of analysis, but has embodied the same in a separate chapter on biological work, since an introduction to such a chapter is necessary before a general idea of cause and effect in this direction can be conveyed. Here will also be found particulars of the microscope, the cultivation of single-cell yeast, the isolation of bacteria, and other matters of general interest.

The author is indebted to Messrs Townsen & Mercer, 34 Camomile Street, London, E.C., and to Messrs Baird & Tatlock, 14 Cross Street, London, E.C., for the loan of blocks from which numerous illustrations have been produced. The reader is assumed to have a slight knowledge of theoretical chemistry, if not of practical analytical methods, but the book is not intended to replace any of the already numerous works on chemistry. Finally, although the work is primarily intended for beginners, it is anticipated that advanced students and qualified analysts will find it useful as a work of reference.

R. DOUGLAS BAILEY, F.C.S., F.R.M.S.

LONDON, *July* 1907.

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THE BREWER'S ANALYST.

PART I.

QUALITATIVE AND QUANTITATIVE ANALYSIS.

ANALYSIS is broadly divided into two classes, qualitative and quantitative, the former consisting in processes for detecting one or more or the whole of the constituents of a substance, the latter in separating out one or more or the whole of the constituents either in a pure state or in the form of some new substance of known composition, and accurately estimating the quantity of the product or products.

There are two methods by which this may be performed, the first being known as

GRAVIMETRIC,

that is to say, separating out the constituents by gravity in the form of a precipitate, collecting and weighing the same; the second method being known as

VOLUMETRIC,

consisting in submitting the substance to be estimated to certain characteristic reactions, employing for such reaction solutions of known strength, and from the volume of solution necessary for the production of such reaction determining the weight of the substance to be estimated by the aid of the known laws of chemical equivalence. Both gravimetric and volumetric methods are adopted in the analytical work described in subsequent pages, and as the accuracy of both depend in the first place upon certain principles, the employment of perfect thermometers, accurately adjusted balances, weights, burettes, pipettes, measuring flasks, etc., of definite capacity, and the use of certain forms of apparatus, we may proceed at once to briefly consider the same.

1. **The Bunsen burner** (fig. 1) is generally employed in the laboratory for heating purposes.

It is so constructed that the coal-gas, before being burnt, is mixed with a proper proportion of air, which is drawn in through holes at the lower part of the burner. The flame is non-luminous, and is smaller than the bright flame. It deposits no soot upon a cool object. Its high temperature, non-luminosity, and colourless appearance also render it very valuable for producing flame colorations. The burner is provided with means for partly or entirely closing the air-inlets when requisite. This is usually effected by turning round a loose perforated ring, which is slipped over the holes.

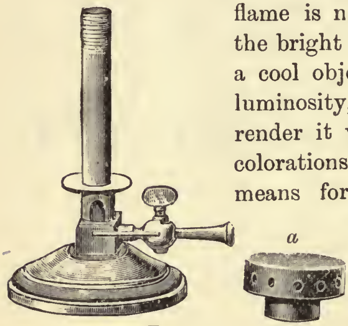


FIG. 1.

When the burner is to be used, it is connected, by means of a piece of tightly-fitting india-rubber tubing, about $\frac{5}{16}$ths of an inch in internal diameter, with the tube which supplies gas to the working bench. The gas-tap is then turned on, and in a few seconds the gas is lighted. The flame should be almost colourless, and give scarcely any light.

When a small flame is used, the supply of air should be partly

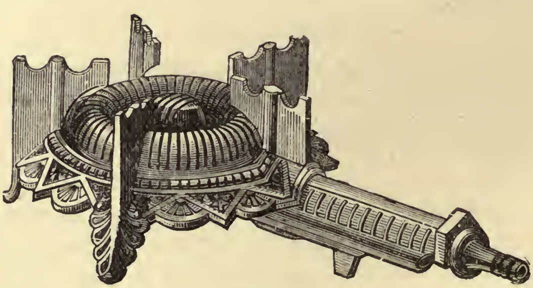


FIG. 2.

shut off, else the flame is apt to recede and *burn below*. If this should occur, the gas supply is stopped by pinching the rubber tube, the supply of air is reduced, and the flame is then relighted. The effect of partially or entirely closing the air-holes of the burner should be learnt by experiment.

For diffusing heat over a large surface, a small perforated metal cap, called the *rose-top* (*a*, fig. 1), is placed upon the top of the

burner. It yields a circle of small flames, and thus diffuses the heat. Other forms of the burner are shown in figs. 2 and 3.

In country laboratories, where gas is not obtainable, a very convenient form of burner is that shown in fig. 4, which is automatic in action and constructed for burning methylated spirit contained in the reservoir.

2. **The spirit-lamp** is occasionally employed instead of the gas or spirit burner; but for general purposes it, or the methy-

FIG. 3.

ated spirit burner, should only be employed when coal-gas cannot be obtained.

The spirit-lamp (fig. 5) consists of a glass vessel containing methylated spirit, into which dips a cotton wick supported by means of a brass or stone-ware wick-holder. When the lamp is not in use, the upper end of the wick should be covered with the glass cap, to prevent evaporation of the spirit.

3. **Glass tube or rod is cut** by making a deep scratch with the edge of a three-cornered file at the point to be cut. The glass is then held with both hands, and a gentle pressure is exerted upon the glass as if trying to break it across. If the file-mark has been made sufficiently deep, the glass will readily break at this point. The sharp edges of a freshly-cut rod or

tube should always be rounded by holding them in the Bunsen flame or blowpipe flame until they are partly melted, or by rubbing them with the face of a file.

4. **Glass tube is bent** by holding it in the upper edge of a common fish-tail gas-flame. The tube is constantly turned slowly round on its axis, so as to heat all sides equally. As soon as the glass is felt to be soft and pliable, it is quickly bent to the required angle. The heated part must not be allowed to touch anything until it is cold. It is then cleansed from soot by means of a cloth.

A bend, if properly made, should be a curve, and should not

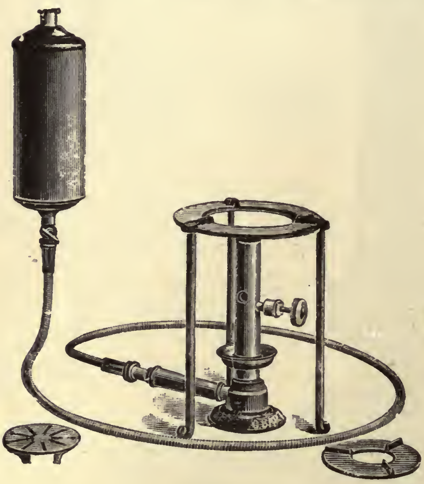


Fig. 4.

Fig. 5.

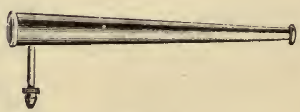


Fig. 6.

alter the bore of the tube. If a sharp angle is made, the bore will be narrowed, and the bend, besides being unsightly, will be very liable to break under a small strain.

Glass rod may be bent in the Bunsen flame or in the blowpipe flame.

5. **The blowpipe** is used for producing a small but very hot flame. This is effected by blowing a fine stream of air through an ordinary flame. The blowpipe (fig. 6) is held in the mouth, and after the cheeks have been blown out to their full extent, the air contained in them is forced out through the jet. This produces a small-pointed, conical flame in the direction of the blast. The chief difficulty in using the blowpipe properly is experienced in maintaining the blast of air uninterrupted by the respiration. The cheeks are kept inflated with air, so that the air

may be forced through the blowpipe by the pressure of the cheeks alone, and not by the action of the lungs. Breathing is carried on meanwhile through the nose; and the cheeks are occasionally replenished with air from the lungs.

It is frequently necessary to have both hands free while the blowpipe is being used. This may be secured by resting the jet on the top of the burner.

A blowpipe which is fed with air from a foot-bellows (fig. 7), or from a mechanical or water blower, is often indispensable for maintaining a high temperature, or for extensive glass-working or glass-blowing.

6. **Small Ignition Tubes.**—A piece of hard glass tubing,

FIG. 7.

five inches long, is drawn out at its middle point by heating it strongly in the blowpipe flame. While the tube is being heated, it is constantly turned round upon its long axis, and when softened, it is gradually drawn out by pulling its ends in opposite directions. By heating the conical parts successively in the blowpipe flame, the narrow tube may be drawn off, and two small closed tubes obtained. If the closed end of the tube is strongly heated in the blowpipe flame, and is then gently blown into while it is red-hot, it may be expanded into a small bulb.

Small test-tubes, three inches long by half an inch in diameter, will also serve for ignition tubes (fig. 8).

7. **Mounted Platinum Wires.**—Two pieces of platinum wire, each about two inches in length, should be fixed in glass handles in the following way:—Draw out a piece of glass tube, five inches

in length, at its middle point, and cut it across at the middle of the narrow portion. Each piece of glass thus obtained serves for the handle of a wire. Break off the narrow part of the tube until it extends only about a quarter of an inch from the shoulder. Insert the end of the platinum wire into this narrow opening; and hold the end of the tube in the blowpipe flame until the glass melts and thickens around the wire, fixing it firmly when cold. Then roll the free end of the wire round a stout wire, so as to shape it into a loop about the eighth of an inch across.

8. **Glass Stirring-Rods.**—Cut some glass rods into lengths of three, six, and seven inches. Heat both ends of each of these rods to redness in the blowpipe flame, the rod being meanwhile constantly turned round on its long axis. The sharp edges are thus removed. The end of the rod must not be allowed to touch anything until it is cool. If a very thin glass rod is required, heat part of an ordinary rod in the blowpipe flame until it is soft, then draw it out to the requisite degree of fineness.

FIG. 8.

FIG. 9.

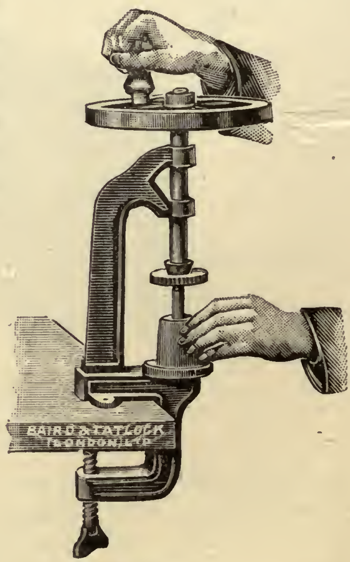


FIG. 10.

9. **Corks are bored** by means of the cork-borer, which is a brass or steel tube sharpened at one end. These are of various sizes, one fitting into another (fig. 9). A borer is selected of slightly less diameter than the glass tube which is to be inserted into the cork. The cork is then pressed against a wooden surface, and the perforation is made by gently pushing the borer through it with a constant movement of rotation upon its axis.

A convenient mechanical contrivance for boring holes through corks and rubber stoppers is shown (fig. 10), several borers of different sizes being supplied which may be conveniently screwed to the apparatus as required.

A slender round file is used for smoothing the interior of the hole made by the cork-borer, and for slightly enlarging it. Great care must be taken to leave the hole round in shape, and not to enlarge it so much that the glass tubing, when inserted, fits loosely. The cork-borer is sharpened by rubbing the outer part of the edge obliquely with the face of a fine-toothed file.

10. **The Wash-bottle.**—A thin, flat-bottomed conical flask, about eighteen ounces in capacity, and with a neck about an inch in diameter, is fitted as is shown in fig. 11.

Select a sound cork which is slightly too large to enter the neck of the flask. Roll it backwards and forwards under the foot with gentle pressure. When the cork has been thus softened, it must fit tightly into the neck of the flask.

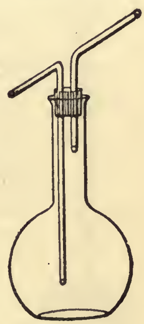


Fig. 11.

Two pieces of glass tubing, rather longer than would be required for the tubes, are then bent (4) into the form shown in fig. 11. Their ends are cut off to the right length, and the sharp edges are rounded (3).

Two parallel holes are then made in the cork by means of a cork-borer (9). The holes must be somewhat smaller than the glass tubes, and may be smoothed, and slightly enlarged, if necessary, by the round file. Into these holes the tubes are then gently pushed; they must enter somewhat stiffly.

An india-rubber stopper *is much more durable than a cork for this and for most other chemical purposes. It may be purchased with two holes already made, or may be perforated by a sharp, wetted cork-borer* (9). *Both the glass tubes and the inside of the holes should be well wetted before the tubes are inserted, since water serves as a lubricant for glass against rubber.* Before the fitting of the flask is proceeded with, insert the cork with the tubes into the neck. Close one tube with the finger, and blow down the other tube. A leakage of air is, as a rule, easily detected; but by wetting the outside of the cork, the escape of air-bubbles becomes visible.

If the cork is air-tight, fit upon the long tube a piece of india-rubber tubing about an inch in length. Into the other end

of this rubber tube push a short jet, made by drawing out a piece of glass tubing in the blowpipe flame. The neck of the flask may then be bound round with twine like the handle of a cricket bat, or tightly covered with a folded strip of rubber or other material. This renders it possible to handle the wash-bottle when it is hot.

The wash-bottle is now nearly filled with distilled water, and is ready for use.

Tap-water should not be kept in the wash-bottle. A fine stream of water may be obtained from the jet by blowing down the short tube. This stream serves for washing percipitates and for other purposes. If a larger stream is required, the flask is inverted, when the water will flow out from the end of the short tube, air entering meanwhile by the long tube.

When hot water is required, the wash-bottle is supported on a tripod stand upon a piece of coarse iron-wire gauze, and is heated by the Bunsen flame, or it may be placed on the water-bath (figs. 29, 30, and 31).

It is preferable to have two wash-bottles, one as described for hot water, the other of stronger build for cold.

FIG. 12.

11. **Cleaning Apparatus.**—It is indispensable to the success of an analyst that all glass and porcelain apparatus should be scrupulously clean, and before beginning to work, the student will do well to clean his set of apparatus as directed below.

Test-tube Brush.—This brush is constantly used for cleaning glass and porcelain apparatus. The piece of sponge ordinarily fastened on the end of the brush does not well adapt itself to the bottom of test-tubes and boiling-tubes. A much more efficient end is given to the brush by removing the sponge and bending back the end of the wire stem sharply upon itself at a point just above where the hairs commence (fig. 12).

Test-tubes, beakers, and porcelain dishes are washed in a stream of tap-water, their surface being rubbed meanwhile by the test-tube cleaner. If the brush fails to remove a stain, hot dilute hydrochloric acid may be used. Sometimes it is necessary to heat a little strong sulphuric or nitric acid in a vessel in order to cleanse it. Hot caustic potash solution may be used to remove grease. In fact, when removing a substance from a vessel to

which it strongly adheres, it should be treated by a liquid in which it is easily soluble. Each article, after it has been carefully washed with tap-water, should be placed upside down in a wicker basket to drain.

Apparatus should be washed as soon as possible after use, since the surface is more difficult to clean after standing.

Test-tubes containing liquids are placed in a test-tube stand. After being washed, they should be placed to drain mouth downwards in the wicker basket.

Platinum foil and wire are cleansed by boiling them in hydrochloric acid and rinsing off the acid with water. The wire should then be strongly heated for some time in the blowpipe flame, until, when wetted with pure, strong hydrochloric acid and held in the Bunsen flame, it no longer persistently colours the flame. If the tip of the wire cannot be cleansed in this way, it should be cut off. Commercial platinum is sometimes

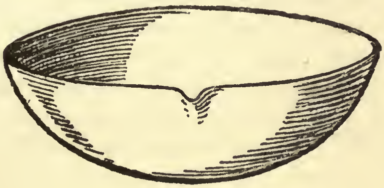


Fig. 13.

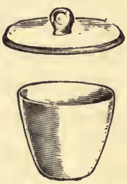


Fig. 14.

alloyed with barium or some other metal which colours the flame. Wire made from such platinum is useless for flame-coloration tests.

Instead of cleaning platinum foil and wire immediately before use, it is better to keep them in a small beaker containing moderately strong hydrochloric acid. The platinum, when removed from the acid and rinsed with water, will then usually be sufficiently clean for use.

Before putting apparatus away, it should be made a rule to wash all glass or porcelain which is not in actual use, and place it in the wicker basket to drain. The basket is then put away with its contents. *Dirty* apparatus should never be kept in the basket. *All iron apparatus* should be carefully dried, and must be kept in a dry place to prevent it from rusting. Metal apparatus must never be put into the wicker draining-basket.

12. **Heating Porcelain and Glass.** — The two following rules must be attended to when either a glass or a

porcelain vessel is being heated, in order to avoid the risk of cracking it.

A vessel containing a liquid must never be heated by the flame above the level of the liquid inside. A dry, hot vessel must be allowed to cool before it is placed on a cool surface, and before any liquid is poured into it.

Porcelain dishes (fig. 13) are generally used for the purpose of boiling or evaporating liquids. They are supported on a pipe-clay triangle or wire-gauze placed upon a tripod or retort-stand. They may be safely heated by a small naked flame.

Porcelain crucibles (fig. 14) are used for containing solid bodies which are to be strongly heated. They are supported in the same way as porcelain dishes. The flame should not at first be allowed to play steadily upon the bottom of the crucible so as to heat it suddenly; but the burner should be constantly moved slightly from side to side until the porcelain is hot. The crucible should be allowed to cool slowly on the triangle before being removed. The hot crucible and cover are handled by means of the crucible tongs (fig. 15).

Glass vessels require to be heated more cautiously than those made of porcelain. A large naked flame must never be allowed to play for any length of time on one part of the glass surface. In heating a test-tube or boiling-tube, this *local heating* is prevented by holding the tube obliquely with the lower part in the flame, and either moving it gently up and down, or constantly turning it round on its axis. Small quantities of liquid may be boiled in a test-tube; but for boiling larger quantities broader *boiling-tubes* are better suited. The risk of burning the fingers by steam is avoided by bending round the neck of the tube a strip of folded paper, and pinching the ends of the strip together, close to the tube.

FIG. 15.

Glass flasks are most safely heated by placing them upon a piece of coarse wire-gauze on a tripod stand. In many laboratories a sand-bath is employed; the flask may then be heated on the sand; it is better still, however, to heat the flask by placing it on the water-bath.

13. **Thermometers** are instruments for measuring temperatures. Owing to the imperfections of our senses, we are unable to measure temperature by the sensation of heat or cold which

they produce in us, and for this reason recourse must be had to the physical actions of heat on bodies. These actions are of various kinds, but the expansion of bodies has been selected as being the easiest to observe.

Liquids are best suited for the construction of thermometers—the expansion of solids being too small, and that of gases too great. Mercury and alcohol are the only liquids now commonly used.

The mercury thermometer is the most extensively used. It consists of a capillary glass tube, at the end of which is blown a bulb—a cylindrical or spherical reservoir. Both the bulb and a part of the stem are filled with mercury, and the expansion is measured by a scale graduated either on the stem itself, or on a frame to which it is or may be attached.

The construction of the thermometer comprises three operations: *the calibration* of the tube, or its divisions into parts of equal capacity; *the introduction of the mercury* into the reservoir; and *the graduation.*

Division of the Tube into Parts of Equal Capacity.—As the indications of the thermometer are only correct when the divisions of the scale correspond to equal expansions of the mercury in the reservoir, the scale must be graduated, so as to indicate parts of equal capacity in the tube. If the tube were quite cylindrical, and the same diameter throughout, it would only be necessary to divide it into equal lengths. But as the diameter of glass tubes is usually greater at one end than another, parts of equal capacity in the tube are represented by unequal lengths of the scale. In order, therefore, to select a tube of uniform calibre, a thread of mercury about an inch long is introduced into the capillary tube, and moved in different positions in the tube, care being taken to keep it at the same temperature. If the thread is of the same length in every part of the tube, it shows that the capacity is everywhere the same; but if the thread occupies different lengths, the tube is rejected, and another one sought.

Filling the Thermometer.—In order to fill the thermometer with mercury, a small funnel is blown on at the top of the tube, and is filled with mercury; the tube is then slightly inclined, and the air in the bulb is expanded by heating the bulb with a spirit-lamp. The expanded air partially escapes by the funnel, and on cooling, the air which remains contracts, and a portion of the mercury passes into the bulb. The bulb is then again warmed, and allowed to cool, a fresh quantity of mercury enters, and so on

until the bulb and part of the tube are full of mercury. The mercury is then heated to boiling—the mercurial vapours in escaping carrying with them the air and moisture. The tube being full of the expanded mercury and of mercurial vapour, is then hermetically sealed at the open end, and when the thermometer is again cooled, the mercury should fill the bulb and a portion of the stem.

Graduation of the Thermometer.—The thermometer being filled, it requires to be graduated, that is, to be provided with a scale to which variations of temperature can be referred. And, first of all, two points must be fixed which represent identical temperatures and which can always be easily reproduced.

Experiment has shown that ice always melts at the same temperature whatever be the degree of heat, and that distilled water under the same pressure, and in a vessel of the same kind, always boils at the same temperature. Consequently, for the fixed point, or zero, the temperature of melting ice has been taken; and for a second fixed point, the temperature of boiling water in a metal vessel under the normal atmospheric pressure. This interval of temperature, that is, the range from zero to the boiling-point, is taken as the unit for comparing temperatures; just as a certain length, a foot for instance, is used as a basis for comparing lengths.

Determination of the fixed Points.—To obtain zero, snow or pounded ice is placed in a vessel in the bottom of which there is an aperture by which water may escape. The bulb or part of the stem of the thermometer is immersed in this for about 15 minutes, and a mark is then made on the tube at the level of the mercury and represents zero.

The second fixed point is determined by suspending the thermometer in an apparatus designed so that water may be boiled and the steam escape under ordinary atmospheric pressure. The thermometer is thus surrounded with vapour, the mercury expands, and when it has become stationary the point at which it stops it marked. This is the point sought for.

The determination of the boiling-point of a centigrade thermometer, viz. 100°, would seem to require that the height of the barometer during the experiment should be 29·92 inches or 760 millimetres, for when the barometric height is greater or less than this quantity, water boils either above or below 100° C. But the point 100° C. may always be exactly obtained by making a suitable correction. For every 27 millimetres' difference in height of the barometer, there is a difference in the boiling-point

of 1 degree. If, for example, the height of the barometer is 778 —that is, 18 millimetres or two-thirds of 27, above 760—water would boil at $100\frac{2}{3}$. Consequently, $100\frac{2}{3}$ would have to be marked, on a centigrade thermometer, at the point at which the mercury stops.

Gay-Lussac observed that water boils at a somewhat higher temperature in a glass than in a metal vessel; and as the boiling-point is raised by any salts which are dissolved, it was assumed to be necessary to use a metal vessel and distilled water in fixing the boiling-point. Rudberg showed, however, that these latter precautions are superfluous. The nature of the vessels and salts dissolved in ordinary water influence the temperature of boiling water but not that of the vapour which is formed. That is to say, if the temperature of boiling water from any of the above causes is higher than 100° C., the temperature of the vapour does not exceed 100° C., provided the pressure is not more than 760 millimetres. Consequently, the higher point may be determined in a vessel of any material, provided the thermometer is quite surrounded by vapour, and does not dip in the water.

Even with distilled water, the bulb of the thermometer must not dip in the liquid; for it is only the upper layer that really has the temperature of 100° C., since the temperature increases from layer to layer towards the bottom in consequence of increased pressure.

Construction of the Scale.—Just as the foot-rule which is adopted as the unit of comparison for length is divided into a number of equal divisions called inches, for the purpose of having a smaller unit of comparison, so likewise the unit of comparison of temperatures, the range from zero to the boiling-point, must be divided into a number of parts of equal capacity called *degrees.* On the Continent, and more especially in France, this space is divided into 100 parts, and this division is called the *Centigrade* or *Celsius* scale, the latter being the name of the inventor.

The degrees are designated by a small cipher placed a little above, on the right of the number which marks the temperature, and to indicate temperatures below zero the minus sign is placed before them. Thus $-15°$ signifies 15 degrees below zero.

In accurate thermometers the scale is marked on the stem itself. It cannot be displaced, and its length remains fixed, as glass has very little expansibility. The graduation is effected by covering the stem with a thin layer of wax, and then marking the divisions of the scale, as well as the corresponding numbers, with

a steel point. The thermometer is then exposed for about ten minutes to the vapours of hydrofluoric acid, which attacks the glass where the wax has been removed. The rest of the wax is then removed, and the stem is found to be permanently etched.

Besides the *Centigrade scale*, two others are frequently used—*Fahrenheit's scale* and *Réaumur's scale.*

In Réaumur's scale the fixed points are the same as on the Centigrade scale, but the distance between them is divided into 80 degrees instead of into 100. That is to say, 80 degrees Réaumur are equal to 100 degrees Centigrade; 1 degree Réaumur is equal to $\frac{100}{80}$ or $\frac{5}{4}$ of a degree Centigrade; and 1 degree Centigrade equals $\frac{80}{100}$ or $\frac{4}{5}$ degrees Réaumur. Consequently, to convert any number of Réaumur's degrees into Centigrade degrees (20 for example), it is merely necessary to multiply them by $\frac{5}{4}$ (which gives 25). Similarly, Centigrade degrees are converted into Réaumur by multiplying them by $\frac{4}{5}$.

The thermometric scale invented by Fahrenheit in 1714 is still largely used in England and elsewhere. The higher fixed point is like that of the other scales, the temperature of boiling water, but the null point or zero is the temperature obtained by mixing equal weights of sal-ammoniac and snow, and the interval between the two points (the lowest then known) was thought to represent absolute cold.

When Fahrenheit's thermometer is placed in melting ice it stands at 32 degrees, and therefore 100 degrees on the Centigrade scale are equal to 180 degrees on the Fahrenheit scale, and thus 1 degree Centigrade is equal to $\frac{9}{5}$ of a degree Fahrenheit, and inversely 1 degree Fahrenheit is equal to $\frac{5}{9}$ of a degree Centigrade.

If it be required to convert a certain number of Fahrenheit degrees (95 for example) into Centigrade degrees, the number 32 must first be subtracted in order that the degrees may count from the same part of the scale. The remainder in the example is thus 63, and as 1 degree Fahrenheit is equal to $\frac{5}{9}$ of a degree Centigrade, 63 degrees are equal to $63 \times \frac{5}{9}$ or 35 degrees Centigrade.

If F be the given temperature in Fahrenheit degrees and C the corresponding temperature in Centigrade degrees, the former may be converted into the latter by means of the formula

$$\left(F - 32\right)\frac{5}{9} = C,$$

and conversely, Centigrade degrees may be converted into Fahrenheit by means of the formula

$$\frac{9}{5}C + 32 = F.$$

These formulæ are applicable to all temperatures of the two scales, provided the signs are taken into account. Thus, to convert the temperature of 5 degrees Fahrenheit into Centigrade degrees, we have

$$\left(5 - 32\right)\frac{5}{9} = \frac{-27 \times 5}{9} = -15 \text{ C}.$$

In like manner we have, for converting Réaumur into Fahrenheit degrees, the formula

$$\frac{9}{4}R + 32 = F,$$

and conversely, for changing Fahrenheit into Réaumur degrees the formula

$$\left(F - 32\right)\frac{4}{9} = R.$$

More briefly stated we have :—

°F. to °C.,	subtract	32,	multiply by 5,	and divide by 9.	
°F. ,, °R.,	,,	32	,, 4	,, 9.	
°R. ,, °F.,	multiply by	9,	divide by 4,	and add 32.	
°R. ,, °C.,	,,	5	,, 4.		
°C. ,, °R.,	,,	4	,, 5.		
°C. ,, °F.,	,,	9	,, 5,	and add 32.	

14. **Balances.**—In a brewer's laboratory, a pair of scales as shown (fig. 16), and weights ranging from $\frac{1}{4}$ oz. to 2 lbs. (fig. 17) will be found extremely useful, especially in weighing barley, malt, hops, sugar, etc., where extreme accuracy is not needed. It is absolutely essential, however, that the laboratory be equipped with a really good light and delicate balance (fig. 18) to carry 100 grams and turn easily and quickly when loaded, with a weight of one- or two-tenths of a milligram (figs. 19, 20). The balance consists of a perfectly rigid metal beam suspended near its centre of gravity on a fulcrum, the substance under comparison being suspended from pivots placed at either extremity of the beam, equidistant from and in the same horizontal line with the fulcrum in the centre. The beam usually rests, by means of a triangular piece of steel termed a knife-edge, on a plate of polished

agate. At the beam-arms similar arrangements exist, the knife-edge in each case being reversed and supporting an agate plate, from which depends a hook, with wires attached to and supporting each pan. A small metal vane is fixed on the exact centre of the

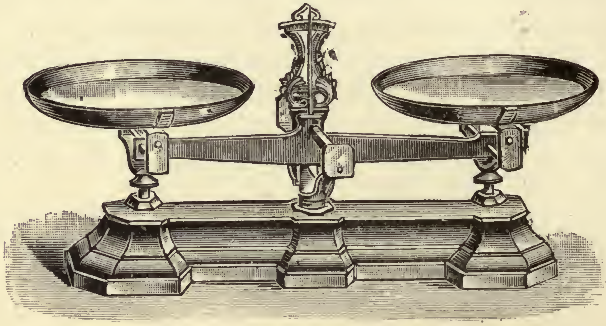


FIG. 16.

beam above the central knife-edge, which, by being turned to the right or left, compensates any inequality in the weight of the arms or pans; or, instead of a vane, a screw weight may be employed at one end of the beam, the precise distance of which, from the centre, may be adjusted as required. The movements of the beam are indicated by a vertical pointer which oscillates before a small ivory scale fixed to the base of the pillar.

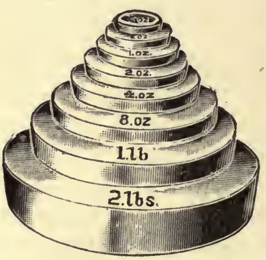


FIG. 17.

A balance of this description serves for weighing small quantities of substances to be tested, many of which are hygroscopic and need to be weighed quickly and with great accuracy. It also serves for testing the accuracy of pipettes and burettes, and, in fact, for the preparation of standard and other solutions and all gravimetric analyses, as mentioned in subsequent pages.

15. **Weights and Weighing.**—The weights most convenient for use in chemical experiments are those based on the decimal or metric system. A box of weights (fig. 19) ranging from 100 grams to a milligram is most useful and sufficient for all chemical purposes. Since these weights are either multiples or sub-multiples of ten, the weight of a substance is most conveniently written down in the decimal notation. Thus a weight of 10 grams, 6 decigrams, 4 centigrams, and 3 milligrams, would be written 10·643 grams.

The larger weights are usually made of brass, the smaller ones of platinum or aluminium (fig. 20). Instead of employing the very small milligram weight, a wide "rider" (a piece of platinum wire in the shape ♌), weighing 1 centigram, is generally used. This is suspended over the graduated scale along the beam of the balance, the scale being graduated in ten divisions, each of which corresponds to 1 milligram. The "rider" placed on the extreme end or tenth division of the scale, immediately over the axis of the pan, equals 1 centigram, or, what is the

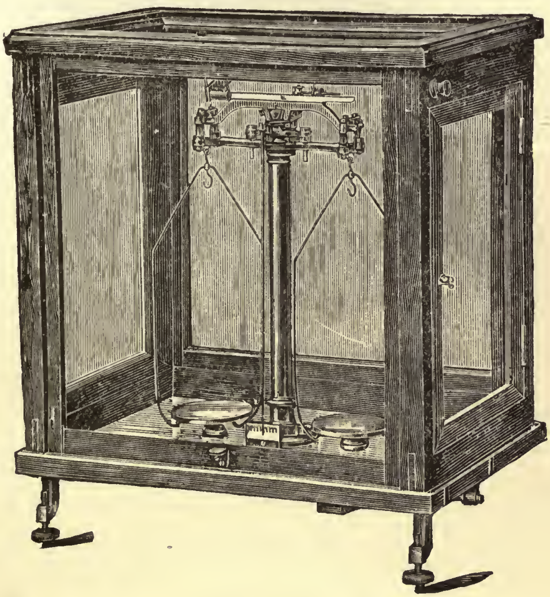


Fig. 18.

same thing, 10 milligrams; placed on the fifth division, it equals but half this amount, and so on. Sometimes the arm of the balance is divided into twelve divisions, in which case the rider weighs 12 milligrams. Each division, of course, then corresponds to 1 milligram.

The following rules should be observed during the process of weighing:—

(*a*) Place the substance on the left-hand pan of the balance and the weights on the right.

(*b*) No substance must be placed directly on the scale-pan.

(*c*) Most substances can be weighed on a watch-glass or in a

small dish, which has been previously weighed, or balanced by means of a counterpoise.

(*d*) A non-volatile liquid is weighed in a balanced beaker or dish; a volatile liquid is weighed in a stoppered bottle.

FIG. 19.

(*e*) The weights should not be handled by the fingers, but should be lifted by a brass forceps.

(*f*) The weights should be placed on the pan in a systematic manner. Commence with the heavier weights, and if they are too heavy, take the lighter weights in succession until the correct ones are found. This method attains the result more quickly and certainly than a random selection of weights would do.

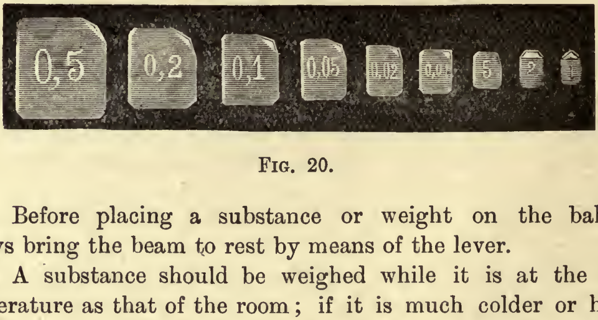


FIG. 20.

(*g*) Before placing a substance or weight on the balance, always bring the beam to rest by means of the lever.

(*h*) A substance should be weighed while it is at the same temperature as that of the room; if it is much colder or hotter than the surrounding air, the body will appear heavier or lighter than it should do.

(*i*) The substance may be considered to be balanced by the weights when the pointer swings through equal distances on both sides of the central mark on the graduated ivory scale fixed at the base of the pillar of the balance.

Occasionally the balance requires a careful removal of its various parts for cleaning. The greatest care should be exercised in this matter; the various parts should be placed on a clean sheet of paper, carefully wiped with a chamois leather, and returned to their positions. Weights may be cleansed in the same manner, and on no account should they be scraped or cleansed otherwise than in the manner directed, excepting the smaller weights, which, if of platinum, may be passed through a smokeless Bunsen flame which will remove any organic matter which may have become attached after prolonged use. The smaller weights, however, are sometimes made of aluminium, in which case they must not be heated.

The balance case should never be left open when out of use, should be closed while ascertaining the final weight of any substance by means of the rider, and neither weights nor any substance should be allowed to remain on the pans for any length of time.

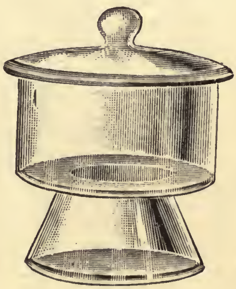


FIG. 21.

16. **The desiccator** usually employed in a laboratory consists of a glass dish of the form shown in fig. 21. In the bottom of the vessel sulphuric acid is placed, and over this is fitted a perforated tray of metal. The rim of the vessel is smeared with grease or vaseline, so that when it is covered by the lid the atmosphere is excluded. Upon placing a substance to be cooled, such as a crucible containing a calcined precipitate, on the perforated tray, and then fixing the lid so as to render the vessel air-tight, the crucible and its contents are, in time, cooled to the ordinary temperature, the sulphuric acid at the bottom of the desiccator absorbing the moisture. Substances should not be weighed until quite cold. Should they have been previously heated, they would, in the ordinary way, have to be exposed for a long period to the atmosphere in order to become cold; and since all bodies, on cooling, attract moisture to their surfaces in varying degree, their weight by reason of this becomes augmented. This applies with greater force to bodies which cool but slowly and to them that readily absorb moisture, so that the use of a desiccator becomes obvious.

Fused chloride of calcium is sometimes employed as a desiccating agent in place of sulphuric acid. For the interior of the balance, calcium chloride is preferable, since, should sulphuric acid be here employed and be accidentally spilt, it would ruin the balance, whereas the spilling of calcium chloride might do but little damage. Calcium chloride is not, however, so good a desiccating agent as sulphuric acid, and should not be employed, on this account, in the ordinary desiccator.

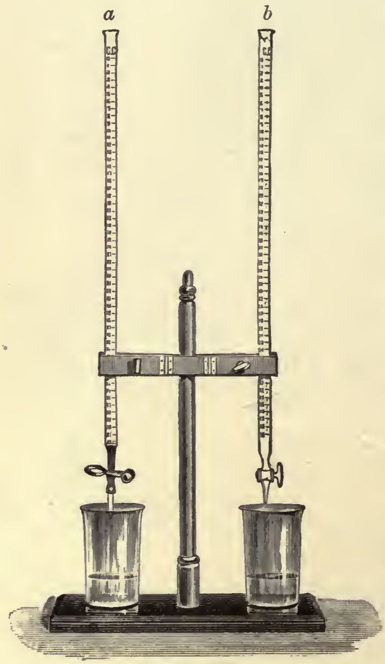


Fig. 22.

17. **Burettes.** — These instruments are used for the delivery of accurately measured quantities of any particular standard or other solution. They invariably consist of a long glass tube of even bore, throughout the length of which are engraved certain divisions corresponding to a known volume of fluid.

They may be obtained in a great many forms under the names of their respective inventors, such as Mohr, Gay-Lussac, Binks, etc. The Mohr burette, with india-rubber tube and metal spring clip (fig. 22, *a*), is preferred to any other, being simple, the quantity of fluid to be delivered being regulated to a nicety by the pressure of the thumb and finger on the clip, and the instrument not being held in the hand, there is no chance of increasing the bulk of the fluid by the heat of the body, and thus leading to incorrect measurement. There is, however, a great drawback to this instrument, viz., it cannot be used for permanganate in consequence of its india-rubber tube, which decomposes the solution ; nor can it be used for iodine or strong alkaline solutions. In such instances it is therefore preferable to employ a burette of the same kind, only fitted with a glass stop-cock, as is shown (fig. 22, *b*).

These instruments are usually of a capacity to hold 100 c.c. or 50 c.c., and graduated in tenths of a c.c.

The burette is filled with liquid from the top, the lower end being closed. A little of the liquid is allowed to flow out through the rubber tube or tap, in order to sweep out air-bubbles. The liquid is then allowed to gradually flow out until the curved under surface, called the meniscus, just reaches the zero graduation near the top of the burette. The appearance of the meniscus is shown in fig. 23. The volume of liquid shown in this figure would be read as 32 c.c.

When the burette is being used, the volume of liquid is read, and the stop-cock is then opened. When the requisite quantity of liquid has flowed out, the reading is again taken. The difference between the two readings will give the volume of the liquid which has flowed from the burette. A light glass float known as Erdmann's float (fig. 24) is sometimes used to facilitate the reading of the liquid volume.

The float consists of an elongated closed glass bulb containing a small quantity of mercury, and having a diameter slightly less than that of the burette; round this bulb is etched a regular line. The position of this line is read off on the burette.

FIG. 23.

FIG. 24.

FIG. 25.

In titrating hot or boiling solutions, the burette should not be arranged directly over the utensil containing the hot liquid, since the burette would be heated and an incorrect reading obtained. It is advisable in such instances to attach to the end of the burette, by means of rubber tubing, a piece of bent glass tubing, so that the heat evolved does not come directly in contact with the burette.

18. **Pipettes.**—A pipette is used for delivering a small volume of a liquid. It usually consists of a tube narrowed at both ends. Two kinds of pipette are in general use: one (fig. 25, *b*) serves to deliver a definite volume of liquid, in this instance 20 c.c.; the other (fig. 25, *a*) is graduated throughout, and serves to deliver fractions of 10 c.c.

In using the pipette, the liquid is drawn into it, by suction, past the graduation mark, and is retained by placing the first finger over the upper end. The pressure of the finger is then slightly relaxed, and the liquid is allowed to flow out until the

lower curve of its surface (meniscus) just reaches the graduation. The finger is then firmly pressed down again so as to arrest the flow. When the liquid is to be "delivered" from the pipette, the finger is removed or its pressure relaxed until the liquid has flowed out. When the pipette is emptied, it is allowed to drain further for a few seconds in a vertical position, so as to deliver the liquid which adheres to its inside. In both the whole and graduated pipettes the upper end is narrowed to about one-eighth inch, so that the pressure of the finger is sufficient to arrest the flow at any point.

The usual capacities are 5 c.c. or 10 c.c. graduated stem; 5 c.c.,

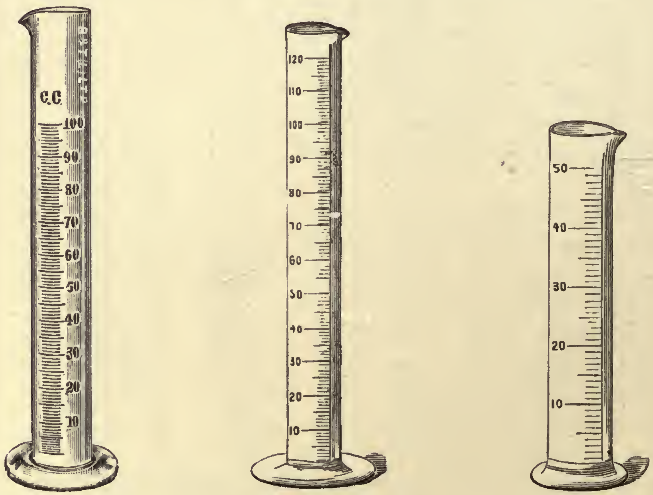


Fig. 26.

10 c.c., 15 c.c., 20 c.c., 25 c.c., 50 c.c., 70 c.c. and 100 c.c. to deliver.

19. **Measuring-Flasks.**—These indispensable instruments are made of various capacities; they serve to mix up standard solutions to a given volume, and also for the subdivision of the liquid to be tested, and are in many ways most convenient. They should be tolerably wide at the mouth, and have a well-ground glass stopper, and the graduation line should fall just below the middle of the neck, so as to allow room for shaking up the liquid.

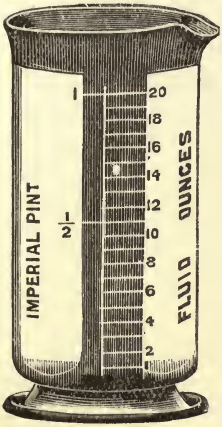
Convenient sizes are 50 c.c. (to deliver), 100 c.c., 200 c.c., 250 c.c., 300 c.c., 500 c.c. and 1000 c.c. (1 litre), all graduated to contain the respective quantities.

Graduated cylinders are shown (fig. 26) and glasses (fig. 27).

The latter are graduated, as shown, to contain from 1 pint to 1 gill and also to show fluid ounces.

Cylinders are usually graduated throughout the greater part of their length, the graduations indicating cubic centimetres, or multiples of cubic centimetres, according to the capacity. They may be obtained either closed by a stopper, or open.

When the measurement of a volume of liquid is being taken,



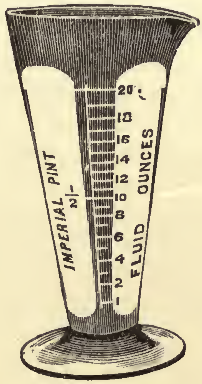


FIG. 27.

the under surface of the liquid (meniscus) should be exactly level with the graduation.

20. **Filtration** is generally performed by employing a glass funnel (fig. 28) into which a folded filter paper is placed, adding the solution to be filtered in such quantity as will not fill the funnel more than three-quarters full at any time.

FIG. 28.

Glass funnels of a diameter of three inches, the sides of which should be perfectly regular and inclined at an angle of 60 degrees, the stems narrow and long and cut off at the end obliquely, are the most convenient to employ.

The liquid to be filtered should be added to the funnel containing the filtered paper, by allowing it to gently run down a glass rod which should be held over the funnel with one hand, the other hand decanting the liquid.

If the liquid were poured into the apex of the paper, loss by spirting might result, and there is the danger also of the paper itself becoming ruptured.

In most cases a portion of the precipitable matter in suspension, or the precipitate itself, when passed on to the filter paper, is apt to pass through the pores of the paper; it is therefore advisable to first see that the pores of the paper are filled before proceeding to use the filtrate. A portion of the liquid to be filtered should therefore be allowed to pass through the paper and then be returned and refiltered. This is in numerous instances essential, whilst in some cases it is necessary to first moisten the filter paper with hot or cold water or weak ammonia before commencing to filter.

The stem of the funnel should be arranged to touch the side of the receptacle receiving the filtrate, so that splashing may be avoided, and the speed of flow accelerated by capillary attraction.

Having transferred the whole of the precipitate to the filter paper, the next operation is to wash it; this is performed by the aid of the wash-bottle (fig. 11). By holding the wash-bottle and blowing through the short tube, the spray of water issuing from the long tube may be directed against the upper edge of the filter paper. Care should be taken, however, to moderate the force of the flow so as to avoid rupturing the paper.

The funnel should not be refilled until the liquid previously added has passed through. A precipitate usually requires three thorough washings after the whole of the liquid has been passed through the paper. This is a point of great importance, as inattention to it is the cause of many worthless results.

Precipitates of a very gelatinous nature take a long time to filter, and the process therefore becomes tedious. In order to accelerate filtration in such instances, one of the simplest and most efficacious ways is to take a rubber stopper through which two holes are bored, fit it to a flask or bottle in the same manner as in constructing a wash-bottle, pass the stem of the glass funnel, through which it is intended to filter, through the hole which would, in the case of the wash-bottle, be fitted with a piece of glass tubing to almost the bottom of the flask, and through the other hole pass the ordinary piece of bent tube. To the end of this bent tube fit a small piece of india-rubber tubing and a metal spring clip. Having inserted the folded filter paper in the funnel and added the liquid it is intended to filter, place the mouth to the end of the rubber tubing and apply suction, by which means the air is withdrawn from the flask and a partial vacuum created; this facilitates the filtration, which, under such circumstances, is wonderfully expedited. In fact, the most gelatinous precipitates can be speedily and effectively filtered in this way. Care should

be exercised to only gently create the partial vacuum, as, otherwise, there is great danger of rupturing the apex of the filter paper.

21. **Evaporation.**—Liquids are easily evaporated by long-continued heating or by boiling them in a porcelain or platinum dish over a Bunsen flame. In these cases, however, there is the liability of spirting, and with such liquids as beer, wort, sugar solutions, etc., there is the disadvantage that their constituents may, by this method of evaporation, caramelise.[1]

It is best to avoid evaporation by direct flame, and in no instance should alcohol, methylated spirit, ether, or liquids containing the same, be subjected to such method of evaporation, as they would undoubtedly catch fire.

Under these circumstances it is wisest in all cases to evaporate liquids over the water-bath.

22. **The water-bath** (figs. 29, 30, 31) consists of a vessel made of sheet copper fitting into another vessel of the same material, leaving a space between the two around the sides and bottom of about 1 inch, and at the top about $1\frac{1}{2}$ inch, so that water may be added to nearly fill the space. There are usually one or two doors in front of the vessel, the interior constituting a drying oven, so that samples of malt, etc., may be introduced and dried. On the top of the vessel there are openings fitted with lids (fig. 29), or a series of flat rings of varying diameter which fit one on top of the other (fig. 30), so that the openings may be made large or small to allow of the exact fitting of the utensil containing the liquid to be evaporated. On the other hand, each opening is fitted with an Iris diaphragm as shown (fig. 31).

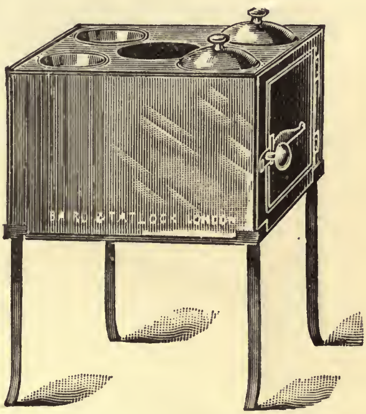


FIG. 29.

A liquid to be evaporated is poured into a porcelain or platinum dish and the dish placed on the water-bath. A Bunsen flame is

[1] In evaporating liquids it should be remembered that both glass and porcelain are attacked by many solutions, and thus error (sometimes serious) may be introduced into analyses. The estimation of alkalies may be affected by this cause. It is preferable, therefore, to employ a platinum dish for all such purposes.

placed beneath the bath, and as the water boils the steam heats the vessel containing the liquid being experimented with, and evaporation is allowed to continue until the liquid is of the bulk desired, or until it is completely evaporated.

In the analysis of malt, when the mash is made and placed on the bath, it is necessary to maintain a constant temperature of 150° F. (65·5° C.) for about an hour. In order to do this the Bunsen burner employed for heating the water in the bath is fitted with a gas regulator, so that the water may be maintained at the proper temperature. The gas regulator is known as the "thermostat."

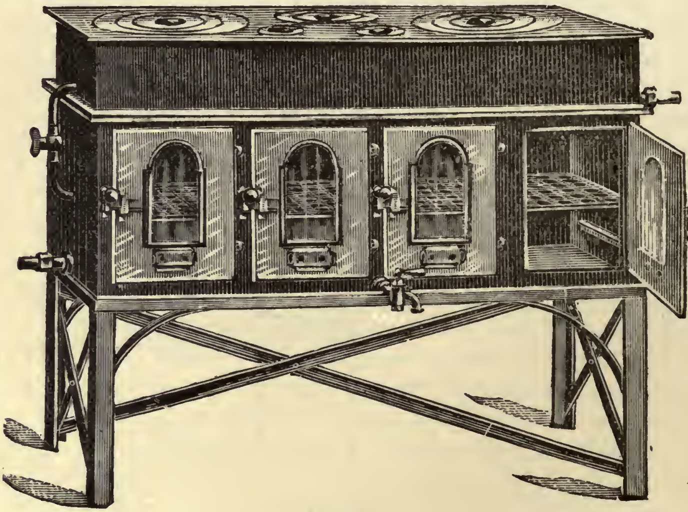


Fig. 30.

23. **Thermostat.**—The principle of this instrument consists in the mercury, when heated, expanding, and closing the main entrance of the gas.

Referring to fig. 32, *a* shows a T-tube ground to fit into the wide part of a thermometer tube; *b* shows a side gas exit tube, and *c* shows a mercury bulb at the foot of the thermometer tube; *s* represents the side tube filled with mercury and fitted with a metal screw; A is a small hole in T-tube *a* opposite *b*. The gas enters by *a* and passes through *b* to the burner. When the temperature rises the mercury expands into the wide part of the cylinder, closing the bottom *a* tube; the burner is thereby reduced, as it is only fed by the small hole in T-tube. S screw regulates the heat. If a high temperature is wanted, then the

mercury must be pressed down the tube to begin with, as it is only when the required temperature is reached that the mercury should rise as high as the widening in the tube, and shut off the excess of gas above that which is required.

Owing to impurities in the gas, the mercury in time becomes coated with a film, which affects the sensibility of the apparatus.

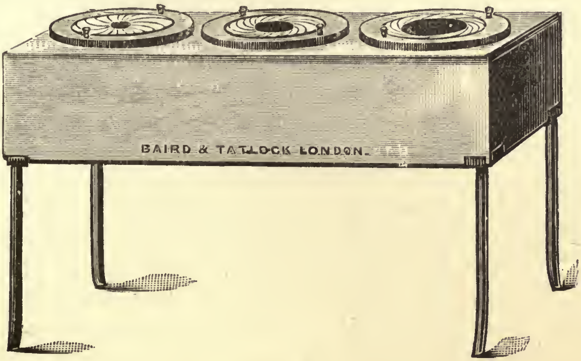


FIG. 31.

In order to remove this film, take out T-tube *a*, screw down the mercury, and clean with a brush.

This regulator, known by the name of its inventor—Reichert—is suitable for all temperatures. For very low temperatures however, instead of a Bunsen burner, it is better to use a burner with a very small opening, almost the size of a blowpipe jet.

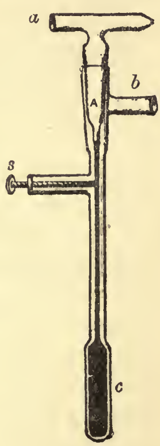


FIG. 32.

24. **The Air-Bath.**—For driving off the water of crystallisation from salts—such as the residue from water—and with substances which do not undergo change at very high temperatures, it is usual to dry them off in an air-bath. This consists of a box of sheet copper having a closely fitting door. It is placed on an iron tripod and heated by a Bunsen flame, the temperature employed and regulated by a thermostat being 215·6° F. (102° C.).

25. **Precipitates and Ignition.**—In some cases it is necessary to weigh a precipitate collected on a filter paper; but before weighing, it becomes essential to thoroughly dry it. In such instances the glass funnel containing the filter paper and its contents is placed in the water oven (22) and thoroughly dried,

after which the paper is removed from the funnel, folded, placed under the desiccator (16) to cool, and afterwards weighed, deducting the weight of the *unburned* paper from the total weight found, whereby we arrive at the weight of the precipitate.

In other cases it becomes necessary to subject the precipitate to ignition. This is performed by folding the paper, placing it in a small *tared* platinum dish or crucible (12), and burning it by means of a small Bunsen flame. In such instances a triangle made of wire, the three sides of which carry small pieces of pipeclay, is employed. This is placed on an iron tripod over a Bunsen flame; the crucible containing the precipitate is then placed on the top, and ignition proceeded with. It is at all times advisable to employ a small flame, and to arrange it so that the tip of its *inner* blue cone approaches to within an eighth of an inch of the bottom of the crucible. Experience has shown, in fact, that inattention to this, such as the application of a large flame which excludes atmospheric air and hence prevents oxidation, may necessitate the ignition of a precipitate for several hours, and that even then the ignition may be imperfect and the precipitate not thoroughly burnt.

With a platinum crucible this would result in considerable damage and loss of weight, thus vitiating the result in a twofold way.

After burning off in the proper manner, the crucible is placed under the desiccator, and when cool, weighed, the weight of the ash of the filter paper being deducted from the weight thus found.

26. **Filter Papers.**—It is always advisable to employ filter papers of the purest kind, that is to say, as free as possible from saline bodies. There are now several kinds on the market, which, when subjected to ignition, leave practically no weighable residue, and obviously they are the best to employ.

The author uses and prefers the Swedish papers manufactured by J. H. Munktell, a box of which contains 500 papers, each of which, when reduced to ash, leaves no weighable residue. With other papers it is necessary to know the amount of ash they leave, so as to deduct this from the weight of a precipitate. The ash will be less, however, when the liquid filtered is acid; for example, when dealing with a precipitate of boric sulphate, the filtrate of which is acid, 1 milligram would probably be the correct difference; whilst for lime, the filtrate of which is alkaline, the same paper would give an ash of 2 milligrams. It is advisable therefore to prepare unknown papers by steeping them over-night in a 5 per cent. solution of hyrochloric acid, afterwards decanting

the liquid and washing the papers with distilled water until the washings are neutral to litmus. Upon then drying the papers in the drying oven, they will invariably be found, upon ignition, to give no appreciable residue.

27. **Distillation.** — There are several forms of distilling or condensing apparatus. Fig. 33 shows Keene's, employed in beer analysis, which consists of a copper tube, curved at the top, to the end of which is attached the distilling flask D. The straight portion of the tube is surrounded by a strong metal jacket through which cold water is passed during distilling, the water entering at A and leaving at B. The distilling flask D is heated by an Argand burner E, and the distillate is collected in a plain or graduated flask C.

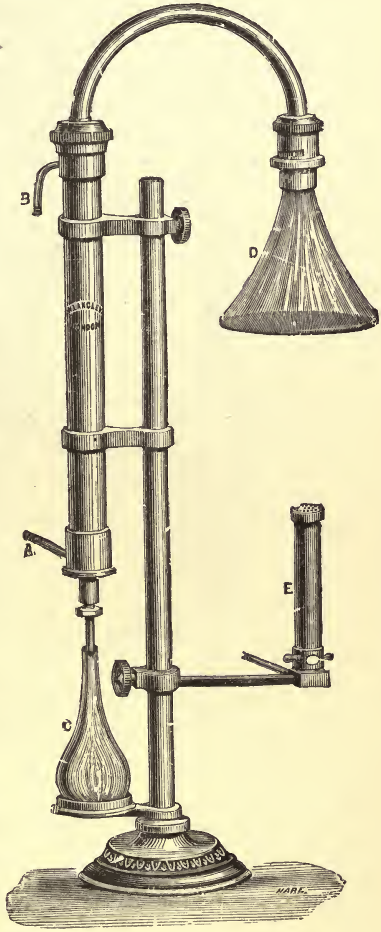


Fig. 33.

Another form of distilling apparatus is Thorpe's (fig. 34), whilst a further form of yet more ancient date is the well-known Liebig's (fig. 35), still employed, particularly in water analysis.

28. **Density and Specific Gravity.**—According to the principle of Archimedes, every body immersed in a liquid is submitted to the action of two forces—gravity, which tends to lower it, and the buoyancy of the liquid which tends to raise it with a force equal to the weight of the displaced liquid. The weight of the body is either totally or partially overcome by this buoyancy, from which it is concluded that a body immersed in a liquid loses a part of its weight equal to the weight of the displaced liquid.

If we take the density of distilled water at a given temperature

to be the unit, the relative density of any other substance is the ratio which the mass of any given volume of that substance at that temperature bears to the mass of an equal volume of water. Thus, it is found that the mass of any volume of platinum is 22·069 times that of an equal volume of water; consequently, the relative density of platinum is 22·069, and this relative density of a substance is called its specific gravity.

In order, therefore, to calculate the specific gravity of a body, it is sufficient to determine its weight and that of an equal

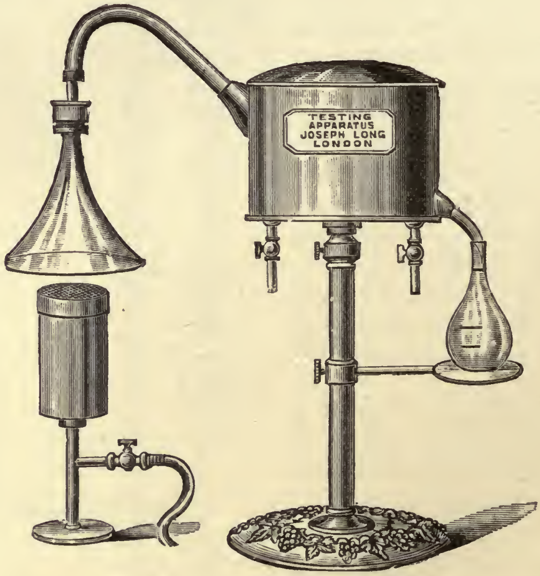


FIG. 34.

volume of water at the same temperature, and then divide the first weight by the second; the quotient is the specific gravity of the body.

In taking the specific gravity of a liquid or solid body, a temperature of 39·2° F. (4° C.) is often chosen, this being the temperature at which water is at its maximum density; for water, unlike most other substances which contract between this temperature and the freezing-point 32° F. (0° C.), expands.

It is exceedingly inconvenient, however, to have to bring bodies to such a low temperature, so in ordinary practice a more readily attainable temperature is chosen, namely 60° F. (15·5° C.).

The specific gravity of gases, however, is determined at the

temperature of the air at the time of performing the experiment, and the volume the particular gas would occupy at any other temperature is found by calculation.

It is obvious that in practical brewing operations a method of arriving at the specific gravity of a solution, requiring a balance, weights and a specific-gravity bottle, is quite out of the question. Instruments were therefore devised which will show the specific gravity of solutions when immersed therein.

These instruments — hydrometers and saccharometers — are well known to the practical brewer; for instance, we have the Sykes hydrometer, the indications of which are simply in degrees of specific gravity over the weight of water. Thus 20° Sykes correspond to 1020° specific gravity. We have also the Bates

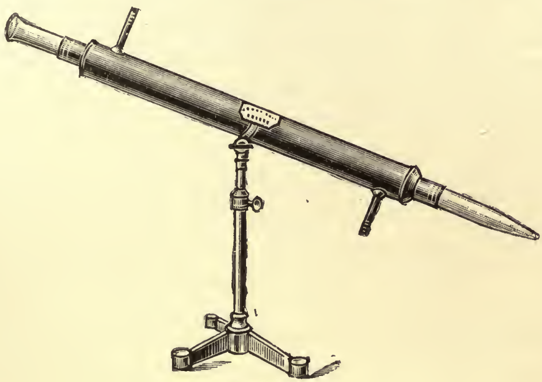


FIG. 35.

hydrometer, which deals with true specific gravity; thus with the smallest counterpoise attached, which is 1000, or equal to the weight of water and floating in a liquid to the graduation mark of 20 on the stem, the specific gravity is 1020°.

We have also the hydrometer of Twaddell, so graduated that real specific gravity may be deduced by a simple method from the degrees indicated, viz., multiplying the latter by 5, and adding 1000; the same being the specific gravity, water being 1000. Thus 5° Twaddell indicate 1025 specific gravity. Again, we have two different instruments under the name of Baumé, one being for liquids heavier than water, the other for those lighter than water, the specific gravity being calculated as follows:—

To convert degrees Baumé into specific gravity, deduct the degree indicated from 144, and then divide 144 by the number so found and multiply by 1000.

Thus to convert 20° Baumé into specific gravity

$$144 - 20 = 124.$$
$$144 \div 124 = 1{\cdot}16129.$$
$$1{\cdot}16129 \times 1000 = 1161{\cdot}29 \text{ specific gravity.}$$

We have also the *saccharometer* of Balling, graduated to indicate the percentage of dry, solid matter in the liquid; and the Dringe and Fage *saccharometer*, graduated to show pounds per barrel. Thus water weighs 10 lbs. to the gallon, so that a barrel of 36 gallons weighs 360 lbs. Should a barrel of beer weigh 380 lbs., the indication by the saccharometer would be 20 lbs, that is, the excess weight over the natural barrel of water.

To convert specific gravity as found by any of the former mentioned hydrometers into pounds per barrel degrees, it is merely necessary to multiply the excess weight over 1000 by ·36 or divide by 2·77. Whilst to convert pounds per barrel degrees into specific gravity, all one has to do is to multiply the pounds per barrel by 2·77 or divide by ·36 and add 1000.

Example:—

1025 specific gravity − 1000 = 25 × ·36 = 9 lbs. per barrel.
1025 ,, − 1000 = 25 ÷ 2·77 = 9 ,, ,,
9 lbs. per barrel × 2·77 + 1000 = 1025 specific gravity.
9 ,, ,, ÷ ·36 = 25 + 1000 = 1025 ,,

Although these instruments are essential in practical operations, they are of little use in analytical work; a pint of liquid is necessary in order to immerse the instrument, and not only is such a quantity seldom at hand in analytical operations, but greater accuracy is necessary than these instruments are capable of showing; hence in analytical operations the specific gravity of a liquid is always ascertained by means of the specific-gravity bottle.

Specific-Gravity Bottle.—This instrument (fig. 36) consists of a glass flask, generally made to contain some even quantity of distilled water at 60° F. (15·5° C.), such as 25, 50, or 100 grams, or 250, 500, or 1000 grains. An accurately ground stopper, perforated by a fine hole, fits into the neck of the flask. The liquid to be weighed is brought to the temperature of 60° F. (15·5° C.), the bottle rinsed with it and then filled, and the stopper fitted, care being taken to exclude air-bubbles. The bottle is then wiped dry with a handkerchief, placed on the balance, and weighed.

The object of the fine hole through the stopper is to allow for

the expansion of the liquid which sometimes takes place owing to the warmth of the atmosphere, or the hands when handling the bottle. The position of the liquid due to expansion thus finds its way up the hole in the stopper, but provided the weighing be quickly performed, the liquid on the top of the stopper will not have had time to evaporate before an exact weight is obtained.

Fig. 36.

Before taking the specific gravity of a liquid, we have first to ascertain the weight of the bottle, both when empty and when filled with distilled water, at 60° F. (15·5° C.); and knowing these two factors, upon weighing any other liquid we obtain the specific gravity by a single calculation.

Example :—

Weight of bottle filled with wort	.	90·960 grams.
Weight of empty bottle . . .	.	21·810 ,,
Weight of wort	.	69·150 ,,
Weight of bottle filled with water	.	86·260 ,,
Weight of empty bottle . . .	.	21·810 ,,
Weight of equal volume of water to that of wort	.	64·450 ,,

$$\frac{\text{Wort } 69{\cdot}150}{\text{Water } 64{\cdot}450} = 1072{\cdot}92 \text{ specific gravity of wort.}$$

The specific-gravity bottle is usually purchased enclosed in a tin box, the lid, which is arranged to hold a small weight, usually containing shot (fig. 36). This weight is a counterpoise for the weight of the empty bottle, so that in the above example the specific-gravity bottle, filled with wort and placed upon the balance, the counterpoise being placed upon the opposite scale pan, would weigh 69·150 grams, which, divided by the weight of water (64·450 grams), which has been previously ascertained and noted so as to save a fresh calculation at each weighing, gives the specific gravity. On the other hand, the specific-gravity bottle may be purchased with a counterpoise, and to contain an exact quantity of liquid such as 100 grams at 60° F. (15·5° C.), the

3

weight of any liquid ascertained by its aid and in the manner directed being, in such case, the specific gravity without any calculation. Specific-gravity bottles, however, as purchased, seldom contain exact quantities; they are nevertheless at times only slightly out, and in such cases, taking it that a bottle when full contains 100·012 grams, with care the stopper may be made smaller by rubbing with fine emery cloth, so as to fit further into the bottle and the bottle thus made to hold exactly 100 grams. In like manner, if the bottle is found to contain less than 100 grams, say for instance 99·997, the bottom of the stopper may be slightly rubbed away with emery cloth so as to allow the extra volume to be retained in the bottle when the stopper is fitted.

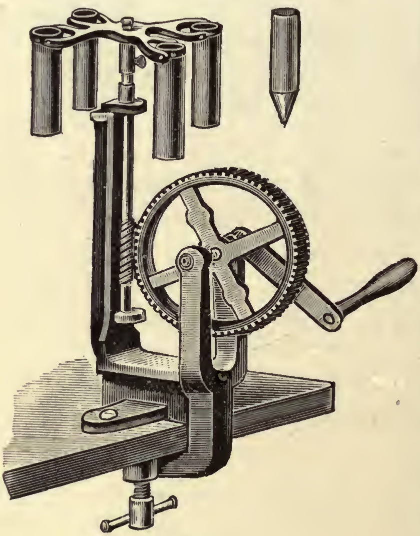


FIG. 37.

29. **The Centrifuge.**—This instrument, more commonly known as the centrifugal machine (figs. 37, 38), has for many years been employed by medical men for the examination of blood and urine, by analysts for bacteriological, microscopical, and other purposes, and particularly for testing milk.

It is employed in the brewer's laboratory for the rapid separation of suspended matter in beer.

The instrument (fig. 37) consists of an upright, which can be clamped on to a table, with four short arms, which can be rotated at extreme velocity by turning the toothed wheel communicating with the screw of the upright. At the end of each arm hangs a metal test-tube, into which slides a glass test-tube holding the beer. When the machine is operated, the pendent tubes rise towards the horizontal position, and the suspended matter in the beer is driven in a compact mass by centrifugal force to the end of the tube. It can then be examined microscopically, biologically, and chemically.

Another form of the instrument is that shown in fig. 38. The test-tubes are closed and placed round the dish in a horizontal

position; the lid is then lowered, and the dish rapidly revolved by means of the strap or a piece of string.

With turbid beers it is often of great importance to know at once the nature of the matter causing the cloudiness. Without the centrifuge it is necessary to allow the suspended bodies to subside—which not only takes time, but may then be incomplete—or we may hasten subsidence by "forcing." Forcing, however, brings about changes other than the mechanical separation of

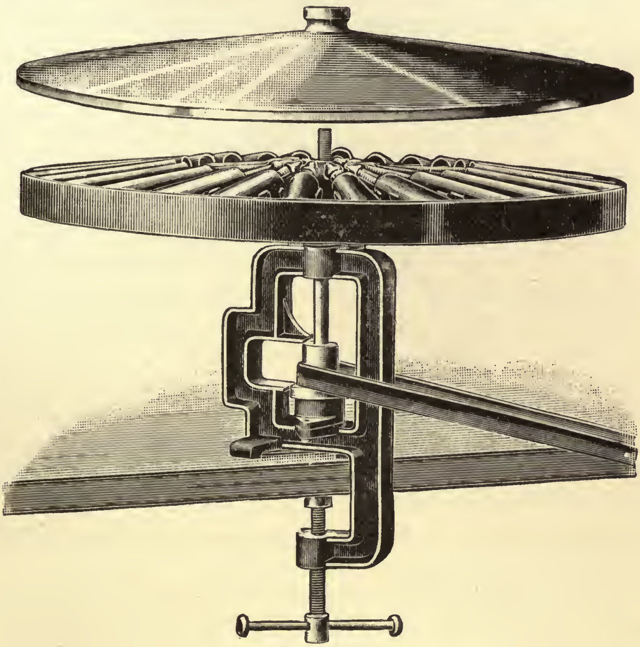


FIG. 38.

suspended bodies, and these are not always required. Where, therefore, it is desired to separate the suspended matter from beers other than by fermentation and putrefactive changes which are induced by forcing, the centrifuge becomes of value, and enables us to gain information as to the nature of the organised and amorphous matters suspended in the beer. Two or three minutes' rotation of the apparatus is usually sufficient for the purpose, but with very stubborn beers (such as those containing budding primary yeast) a slightly longer time is often necessary.

It was shown by H. Tikes [1] seven years ago that micro-organisms

[1] *Allgem. Zeitschr. Bierbrau. und Malzfabrik.*, 1900, 28, 1–2.

can be rapidly separated from beer and other liquids by means of the centrifuge, and it is due to him that the instrument has become a valuable adjunct to the brewer's laboratory.

Sterilised solutions containing equivalent amounts of potash alum and sodium carbonate were prepared, and a small quantity of each added to the liquid to be treated. After the evolution of the carbon dioxide the mixture was spun in a centrifuge until the gelatinous precipitate collected at the bottom of the tube.

The supernatant liquid was then poured off and the precipitated alumina was then dissolved in 1 c.c. caustic soda, which had practically no effect on the bacteria present, and the solution examined under the microscope. By this process it was possible

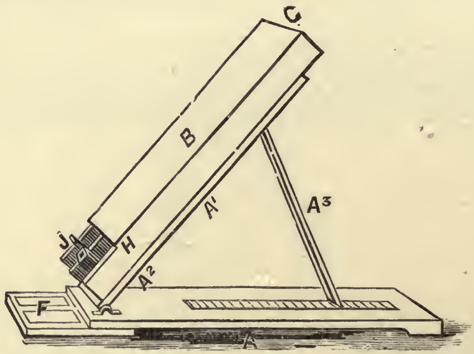


FIG. 39.

to remove 89–90 per cent. of the organisms from beer and 96–100 per cent. of added organisms from water.

30. **The Tintometer.**—This well-known instrument (fig. 39) consists of a binocular tube B, terminating at the object end H in two small square holes. The instrument is arranged to be raised or lowered on the stand A and fixed in position as shown, A³. At F a square of opaque unglazed porcelain is fixed. Supplied with the instrument for brewers' purposes are two cells or metal boxes, fitted at opposite sides with glass, one being known as the 1-inch cell, the other the ½-inch cell. A sample of beer, caramel, sugar, or other solution to be tested is placed in one of the cells, and the cell is then placed in position on the left side of the instrument H. Upon looking through the tube at C the colour of the solution is distinctly seen, and the object now is to match the colour. For this purpose a series of yellow glasses, known as the 52 series, are supplied with the instrument. These glasses range from a decimal or from 1 degree in equal gradation

up to any desired number. For instance, glass number 2 is of double the colour intensity of glass number 1, or glasses numbered 4 and 6 equal in intensity the colour glass number 10. These glasses are taken and slipped into the instrument at J, so that upon looking through the tube at C the colour of both the glasses and the solution are viewed, the glasses being added until the colour of the solution is matched.

The numbers of the glasses are then read and added together, and the colour value expressed as of so many degrees. For example, if glasses 8, 11, and 12 were required to match the colour of the solution in a 1-inch cell, the colour would be registered as equal to 31° in 1′ cell. The author carried out a series of experiments with this instrument in 1888, almost as soon as it was introduced, and may here, with slight alteration, repeat the same as then published.[1]

. . . . The colour of beer is of great importance, since uniformity in this direction is greatly admired by most consumers. In order, therefore, to turn out beers day by day of constant colour uniformity in accordance with their respective quality, some form of instrument is useful, and we here refer to Lovibond's Tintometer.

This instrument answers a chain of questions which are interesting and necessary to the brewer, beginning with the colour of a percentage solution of malt in relation to that of the worts before and after boiling, to the registration of the colour of the beer. A few estimations of colour in malt will lead to a practical knowledge of character and evenness in drying; it will soon be ascertained what colour in malt produces a given colour in ale, and what quantity of black malt or caramel is required to produce a beer of definite colour intensity. Experiments carried out with the tintometer were as follows:—

	Degrees of Colour. 1-inch cell.
Pale malt, 5 per cent. solution	3
Wort before boiling, 1050 specific gravity	10
Wort after boiling	14
Finished beer	13
Finished beer after storing 3 weeks	11

The above are the observations of an actual brewing, the ultimate eleven degrees of colour being about that of the Burton pale ale.

The instrument is also of great value in estimating roasted

[1] *Brewers' Guardian*, Feb. 21, 1888.

malts and caramels, where the value bought is primarily colour, the variations between samples very similar in outward judgment being sometimes very wide :—

Roasted Malt.

			Degrees of Colour.
No. 1.	Black,	1 per cent. solution $\frac{1}{8}$ thickness	$7\frac{1}{4}$
No. 2.	Chocolate,	,, ,, ,,	10

These observations show the large difference in colour value between two samples of which the difference in price was very trifling.

PART II.

THE POLARIMETER.

Polarisation of Light—Specific Rotatory Power—Solution Weight and Solution Factors—Cupric Oxide Reducing Power.

Some twenty years ago the Polarimeter, or "Polariscope" as it was then incorrectly called, was looked upon and described by those who had but little, if any, knowledge of its use, as a "pretty but useless chemical toy." With the rapid strides that have since been made in the science of brewing, the polarimeter has become an indispensable instrument, and has played a wonderful part in enabling those who have worked upon starch-conversion products to define many points, particularly with regard to the composition of the so-called malto-dextrins.

That the polarimeter is a most useful apparatus to the brewer, and one which, without any laborious or tedious experimenting, enables him to estimate the conversion products of his mashes, apart from its value in numerous other well-known directions, is now a recognised fact. As, therefore, it is essential that the modern brewer should become familiar with the instrument, it is necessary that we sketch the principle upon which it is based, and then describe its construction.

The principle of the instrument depends upon the polarisation of light.

Polarisation of Light.—There are four processes by means of which a ray of light may be polarised: these are reflexion, ordinary refraction, double refraction, and scattering by small particles.

It will be convenient, however, to reproduce the description of the phenomena of polarisation as given by Spottiswoode,[1] who

[1] *Polarisation of Light*, Macmillan & Co.

starts with a plate of crystal called tourmalin as an instrument, tolerably simple in its action and easy of manipulation :—

"Tourmalin is a crystal, of which there are several varieties, differing only in colour. Very dark specimens generally answer the purpose well, excepting that it is difficult to cut them thin enough to transmit much light. Red, brown, or green specimens are usually employed; the blue are, for the most part, optically unsuitable. Some white, or nearly white, specimens are very good, and may be cut into thicker plates without loss of light.

"If we take a plate of tourmalin, cut parallel to a particular direction within the crystal called the optic axis, and interpose it in the path of a beam of light at right angles to the direction of the beam, the only effect perceptible to the unassisted eye will be a slight colouring of the light after transmission, in conse-

FIG. 40.

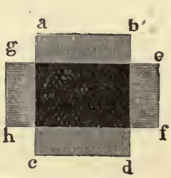


FIG. 41.

quence of the natural tint of the particular piece of crystal. But if we examine the transmitted beam by a second similar plate of tourmalin placed parallel to the former, the following effects will be observed: When the two plates are similarly placed, *i.e.* as if they formed one and the same block of crystal, or, as it is technically expressed, with their optic axes parallel, we shall perceive only, as before, the colouring of the light due to the tints of the two plates. But if either of the plates be then turned round in its own plane so as always to remain perpendicular to the beam, the light will be observed to fade gradually until, when the moving plate has been turned through a right angle, the light becomes completely extinguished. If the turning be continued beyond the right angle, the light will begin to revive; and when a second right angle has been completed, the light will be as bright as at the outset. In figs. 40 and 41, *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h* represent the two plates. In fig. 40 the two plates are supposed to be in the first position; in fig. 41 the plate *e*, *f*, *g*, *h* has been turned through a right angle.

"Of the parts which overlap, the shading in fig. 40 represents

the deepened colour due to the double thickness of the crystal; in fig. 41 it indicates the complete extinction of the light. The same alternation of brightness and extinction will continue for every right angle through which the moving plate is turned. Now it is to be observed that this alternation depends only upon the angle through which one of the crystals has been turned, or, as it is usually stated, upon the relative angular position of the two crystals. Either of them may be turned, and in either direction, and the same sequence of effect will always be produced. But if the pair of plates be turned round bodily together, no change in the brightness of the light will be made. It follows, therefore, that a ray of ordinary light possesses the same properties all round; or, as it may be described in more technical language, a ray of ordinary light is symmetrical in respect of its properties about its own direction. On the other hand, a ray of light, after traversing a plate of tourmalin, has properties similar, it is true, on sides or in directions diametrically opposite to one another, but dissimilar on intermediate sides or directions. The properties in question vary, in fact, from one angular direction to another, and pass through all their phases, or an entire period, in every angle of 180°. This directional character of the properties of the ray, on account of its analogy to the directional character of a magnet or an electric current, suggested the idea of polarity; and hence the condition in which the ray was found to be was called polarisation.

"Having so far anticipated the regular order of things on the experimental side of the subject, it will be worth while to make a similar anticipation on the side of theory. It is considered as established that light is due to the vibrations of an elastic medium, which, in the absence of any better name, is called ether. The ether is understood to pervade all space and all matter, although its motions are affected in different ways by the molecules of the various media which it permeates. The vibrations producing the sensation of light take place in planes perpendicular to the direction of the ray. The paths or orbits of the various vibrating ethereal molecules may be of any form consistent with the mechanical constitution of the ether; but, on the suppositions usually made, and none simpler have been suggested, the only forms possible are the straight line, the circle, and the ellipse. But in ordinary light the orbits at different points of the ray are not all similarly situated; and although there is reason to believe that in general the orbits of a considerable number of consecutive molecules may be similarly situated, yet in a finite portion of the

ray there are a sufficient number of variations of situation to prevent any preponderance of average direction.

"This being assumed, the process of polarisation is understood to be the bringing of all the orbits throughout the entire ray into similar positions. And in the case of the tourmalin plate the orbits are all reduced to straight lines, which consequently lie in one and the same plane. For this reason the polarisation produced by tourmalin, as well as by most other crystals, is called rectilinear, or, more commonly, plane polarisation. This property of tourmalin may also be expressed by saying that it permits only rectilinear vibrations parallel to a particular direction, determined by its own internal structure, to traverse it.

"Adopting this view of polarisation as effected by a plate of tourmalin, it would be interesting to ascertain the exact direction of the vibrations. And a simple experiment will go far to satisfy

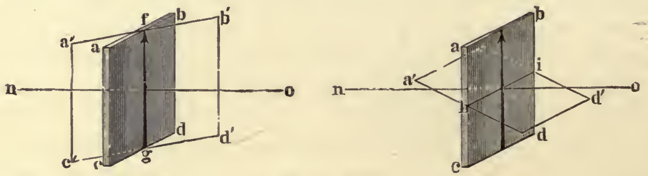


FIG. 42.

us on that point. The argument, as now stated at least, is perhaps based upon general considerations rather than upon strict mechanical proof; but the experimental evidence is so strong that it should not be denied a place here. Suppose for a moment that the tourmalin be so placed that the direction of vibration lies either in or perpendicular to the plane of incidence (that is, the plane containing the incident ray, and perpendicular to the surface on which it falls at the point of incidence); then it is natural to expect that vibrations executed in the plane of incidence will be far more affected by a change in the angle of incidence than those perpendicular to that plane. In fact, the angle between the direction of the vibrations and the surface upon which they impinge will in the first case vary with the angle of incidence, but in the second case it will remain unchanged. In fig. 42, *n o* represents the ray of light; the arrow the direction of vibration; *a*, *b*, *c*, *d*, *a'*, *b'*, *c'*, *d'* the plate in two positions, turned in the first instance about the direction of vibration, in the second about a line perpendicular to it.

"Dismissing, then, the former supposition, and supposing that nothing whatever is known about the direction of vibration, then,

if all possible directions be taken in succession as pivots about which to tilt or turn the second tourmalin, it will be found that for one direction the intensity of the light diminishes more rapidly with an increase of tilting (or, what is the same thing, with an increase of the angle of incidence) than for any other. And, further, that for a direction at right angles to the first, the intensity of light diminishes less than for any other, while for intermediate directions the diminution of intensity is intermediate to those above mentioned. In accordance, therefore, with what was said before, we may conclude that the vibrations are parallel to the line or pivot about which the plate was turned when the diminution of light was least.

"Secondly, polarisation may be effected by reflexion. If light reflected from the surface of almost any, except metallic, bodies be examined with a plate of tourmalin, it will in general be found to show traces of polarisation; that is to say, if the plate of tourmalin is caused to revolve in its own plane, and the reflected rays be viewed through it, then in certain positions of the plate the reflected light will appear less bright than in others. If the angle at which the original rays fall upon the reflecting surface be varied, it will be found that the amount of alteration in brightness of the light seen through the revolving tourmalin (or analyser) will also vary. This fact may also be expressed thus: In polarisation by reflexion, the degree of polarisation, or the amount of polarised light in the reflected rays, varies with the angle of incidence on the reflecting surface. But at a particular angle, called on that account the polarising angle, the polarisation will be a maximum. This angle (usually measured between the incident ray and perpendicular to the reflecting surface) is not the same for all substances; in fact, it varies with their refractive power according to a peculiar law, which may be thus enunciated: The tangent of the polarising angle is equal to the refractive index. Simple geometrical considerations, combined with the usual expressions for the laws of reflexion and refraction, will show that this relation between the polarising angle and the refractive index may be also expressed in the following way: If light be incident at the polarising angle, the reflected and refracted rays will be at right angles to one another.

"In fig. 43, *s i* represents the incident, *i f* the reflected, and *i n* the refracted ray. Then *s i* will be incident at the polarising angle when the angle *f i n* is a right angle.

"An apparatus devised by Tyndall for experimentally demonstrating the laws of reflexion and refraction is admirably adapted

for verifying this law. The following description is quoted from his *Lectures on Light*:—'A shallow circular vessel R I G (fig. 44), with a glass face, half filled with water rendered barely turbid by the admixture of a little milk or the precipitation of a little mastic, is placed upon its edge, with its glass face vertical. By means of a small plane reflector M, and through a slit I in the hoop surrounding the vessel, a beam of light is admitted in any required direction.'

"If a little smoke be thrown into the space above the water, the paths of the incident, the reflected, and the refracted beams will all be visible. If, then, the direction of the incident beam be so adjusted that the reflected and the refracted beams are at right angles to one another, and a Nicol's prism be interposed in

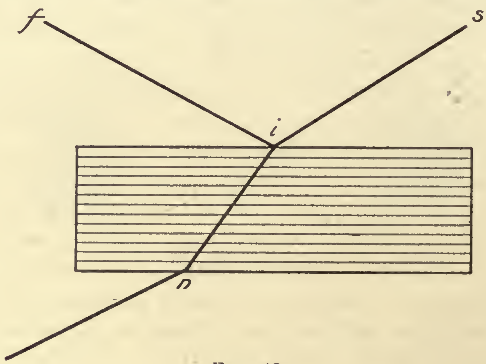


FIG. 43.

the path of the incident beam, it will be found that, by bringing the vibrations alternately into and perpendicular to the plane of incidence, we shall render the intensity of the reflected and refracted rays alternately a minimum.

"Thus much for the verification of the law. But not only so; if we take different fluids, and for each of them in succession adjust the incident beam in the same manner, we shall only have to read off the angle of incidence in order to ascertain the polarising angle of the fluid under examination.

"The general theory of the reflexion and refraction of polarised light was first established by Fresnel, who based it upon the four following suppositions:—

"(1) That the ether, to the vibrations of which light is supposed to be due, is regarded as perfectly elastic, so that the whole of the motion taking place at the source of light is transmitted without loss throughout the ray. This appears to be substantially

true in transparent media; but in proportion as a substance is more opaque, so do its molecules take up part of the motion of the ether, and convert the light into heat (the principle of *vis viva*).

"(2) That in passing from one medium to another, although the velocity and extent of the motion may change, yet its character is not altered (the principle of continuity).

"(3) That any change in velocity or in extent, due to the passage from one medium to another, takes place immediately at the surface of separation; and that such change is maintained subsequently.

"(4) That while the elasticity of the ether in different bodies remains the same, its density may differ.

"By means of these suppositions relations were established between the intensity of the reflected and refracted rays on the one hand, and the angles of reflexion and refraction on the other, from which many phenomena previously known only as experimental facts were deduced as consequences. Of these, one should be mentioned, viz., that in the case of vibrations in the plane of incidence, if the ray be incident at such an angle that the reflected and refracted rays are perpendicular to one another, there can be no reflected ray.

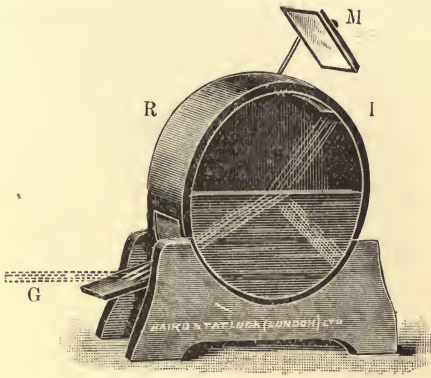


FIG. 44.

"We next come to the subject of polarisation by double refraction.

"There are a large number of crystals which have the property of generally dividing every ray which passes through them into two. But the extent of separation of the two rays varies with the direction of the incident ray in reference to the natural figure of the crystal. In every double refracting crystal there is at least one, and in many there are two, directions in which no separation takes place. These directions are called optic axes.

"Of such crystals Iceland spar (crystallised carbonate of lime) is the most notable instance. If we take a block of such spar

split into its natural shape, a rhombohedron, fig. 45, and for convenience cut off the blunt angles by planes perpendicular to the line joining them, $a\,b$, it will be seen that a ray of light transmitted perpendicularly to these planes, that is, parallel to the line joining the blunt angles, is not divided. In fact, the image of an object seen by the eye in the direction in question appears single, as if passed through a block of glass. The direction in question (viz. the line $a\,b$ itself, and all lines passing through any part of the crystal parallel to $a\,b$) is called the optic axis of the crystal.

"If, however, the crystal be tilted out of this position in any direction, it will be seen, by the appearance of two images instead of one, that the rays are divided into two. The angular divergence of the two sets of rays, or, what comes to the same thing, the separation of the two images, depends upon the angle through

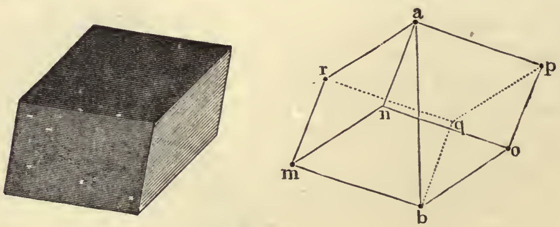


FIG. 45.

which the crystal has been turned; or, as it may also be expressed, upon the angle between the directions of the incident ray and the optic axis of the crystal. When this angle amounts to a right angle the separation is at its greatest; and if the crystal be still further turned, the images begin to come together again until, when it has turned through another right angle, they coincide.

"This process of separation, or doubling the rays, is called double refraction, and the character of the polarisation of two images is best studied by using flat instead of curved surfaces for separating the rays.

"For the purpose in question there is, perhaps, no better instrument than the double-image prism. This consists of a combination of two prisms,—one of Iceland spar, so cut that the optic axis is parallel to the refracting edge; the other of glass, and usually having a refracting angle equal to that of the spar.

"The rays passing through the crystal prism being perpendicu-

lar to the optic axis, undergo the greatest separation possible. And the chromatic dispersion caused by that prism is usually corrected or neutralised entirely in the case of the extraordinary, and nearly so in that of the ordinary, ray by the glass prism, which is placed in a reverse position. In this arrangement the extraordinary image occupies the centre of the field, and remains fixed when the double-image prism is made to revolve in a plane perpendicular to the incident rays; while the ordinary image is diverted to a distance from the centre, and revolves in a circle about that centre when the prism revolves.

"Other dispositions of the double-image prism are also made for particular purposes; *e.g.* in which neither image is central, and in which the chromatic dispersion of both images is partially corrected. If the nature of the light in the two images thus formed be examined by any polarising instrument, it will be found that it is polarised in both cases, and that the vibrations in the one image are always perpendicular to those in the other. And in particular, the vibrations in the extraordinary image are parallel and those in the ordinary are perpendicular to the optic axis.

Fig. 46.

"On these principles polarising and analysing instruments have been constructed by various combinations of wedges or prisms of Iceland spar, the details of which it is not necessary to describe in full. But the general problem and object proposed in all of them has been to cause such a separation of ordinary and extraordinary rays that one set of rays may, by reflexion or other methods, be further diverted, and afterwards thrown altogether out of the field of view. This done, we have a single beam of completely polarised light, and a single image produced from it.

"One such instrument, however, the Nicol's prism, named after its inventor, a clever optician, on account of its great utility and its very extensive use, and which is to be found in all polarimeters, must be described. A rhombohedron of Iceland spar, double of its natural length, is taken (fig. 46); and one of its terminal faces P, which naturally makes an angle of 71° with the blunt edges K, is cut off obliquely so as to give the new face, say P′, an inclination of 68° to the edges K. The whole block is then divided into two by a cut through the angle E in a direction at right angles to the new face P′; the faces of this cut are then carefully polished, and cemented together again in their original

position with Canada balsam. Fig. 47 represents a section of such a prism made by a plane passing through the edges K. A ray entering as *a b* is divided into two, viz. *b c* the ordinary and *b d* the extraordinary. But the refractive index of the Canada balsam is 1·54, *i.e.* intermediate between that of the spar for the ordinary (1·65) and the extraordinary (the minimum value of which is 1·48) rays respectively; and in virtue of this the ordinary ray undergoes total reflexion at the surface of the balsam, while the extraordinary passes through and emerges ultimately parallel to the incident ray. Fig. 48 shows an end view of a Nicol's prism P P, representing the plane of polarisation of the emergent polarised ray.

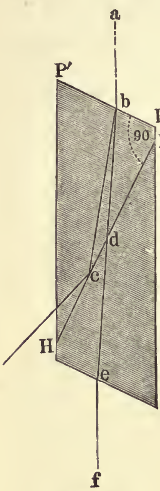


FIG. 47.

"Two such instruments, when used together, are respectively called the "polariser" and the "analyser," on account of the purposes to which they are put. These, when placed in the path of a beam of light, give rise to the following phenomena, which are, in fact, merely reproductions in a simplified form of what has gone before.

"When polariser and analyser are placed in front of one another with their shorter diagonals parallel, that is, when the vibrations in the image transmitted by the one are parallel to those in the image transmitted by the other, the light will be projected exactly as if only one instrument existed. If, however, one instrument, say the analyser, be turned round, the light will be seen to fade in the same way as in the case of the tourmalin plates; until, when it has been turned through a right angle, or, as it is usually expressed, when the polariser and analyser are crossed, the light is totally extinguished.

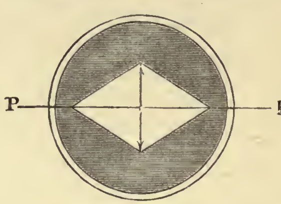


FIG. 48.

"In the complete apparatus or polarimeter we may incorporate any system of lenses, so that we may make use of either parallel or convergent light, and finally focus the image produced."

Now various substances have the property of rotating polarised light, as, for example, turpentine and sugar solutions of various kinds, such as dextrin, maltose, dextrose, etc. If, therefore, we fill a glass tube with turpentine or with a certain percentage sugar

solution, place the tube in the polarimeter and then look through the telescope, it will be noticed that the two halves of the field are unequally illuminated, being darker on one side than on the other. By now adjusting the instrument so that both halves of the field are evenly illuminated, the exact angle of rotation can be read off from the vernier scale. Of the various substances which are optically active, some turn the polarised ray to the right, and are known as right-handed, dextro-rotatory, or positive substances, whilst others bend the ray to the left, and are known as left-handed, lævo-rotatory, or negative bodies; the signs + and − being used to briefly designate each kind. All solutions of an optically active substance of the same strength, when traversed by the polarised ray in layers of the same thickness, rotate the ray, each substance having a definite power. When this is calculated upon a certain definite strength of solution, and upon a layer of such solution of a definite thickness, an expression is found which is termed the "specific rotatory power" or the "opticity" of the substance. The function, then, of the polarimeter is to determine the amount of rotation which the polarised ray suffers in passing through a layer of the solution of an optically active substance.

From this, by taking certain other factors into account, the specific rotatory power of the substance can be found; and when the specific rotatory power of a substance present in solution is known, the polarimeter enables us to estimate its amount.

It is not within the scope of this work to describe the subject at greater length; suffice it to say that the polarisation of light is undoubtedly one of the most intricate but at the same time most beautiful branches of the science of optics. For full information the reader should consult the latest work, viz. *The Optical Rotation of Organic Substances*, by Professor Landolt.[1]

The Polarimeter.—There are several differently constructed polarimeters, such as the Soleil or Ventzke-Scheibler; the Lippich; Laurent; Schmidt & Haensch Half-Shadow instrument, and others; but the two most commonly used forms are the Laurent and Schmidt & Haensch Half-Shadow. With the latter a white light illumination from oil or gas is employed, but with the former a sodium flame illumination is used.

Specific rotatory power, as determined in reference to the D ray of the solar spectrum (that is, with the sodium flame), is indicated by $[\alpha]_D$, whilst when determined by the older forms

[1] Translated from the German by Dr Long. The Chemical Publishing Co., Easton, Pennsylvania.

of instrument, such as the Ventzke-Scheibler, it is indicated by $[a]_j$.

In the Laurent polarimeter which is shown, fig. 49, F indicates the telescope, *l l* the magnifying glasses, *n n* the two verniers, K the graduated dial, A a prism of Iceland spar known as the "analyser," or analysing Nicol, which is fixed to the revolving dial K and to the telescope, and capable of being rotated on its axis by means of the milled head T seen underneath it. It carries with it the two verniers *n n*, which indicate the observed rotation on the scale of the dial-plate K.

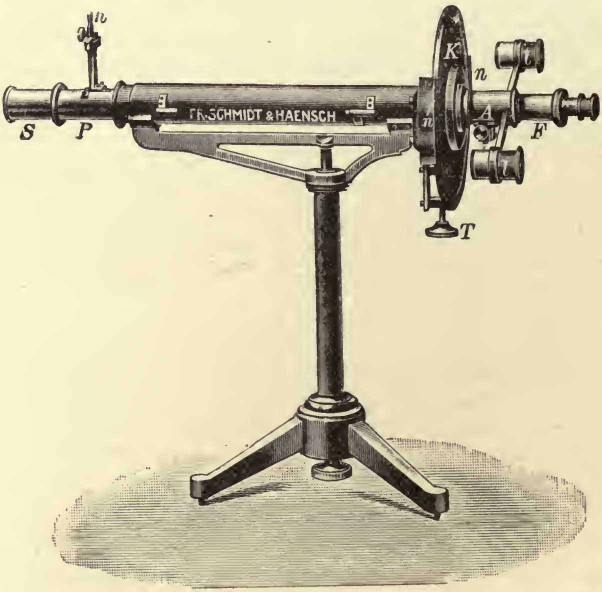


FIG. 49.

Next comes the trough B E, with a lid, in which the tube (fig. 50) filled with the solution to be examined is placed.

P is the movable "polariser" (another prism of Iceland spar), which converts one half of the ordinary light passing through it into polarised light, the other half being reflected to the side and absorbed. Above it is a fixed graduated segment of a circle *h*. At G is a thin plate of quartz, covering half the field, known as Laurent's plate, its function being to increase the delicacy of the indications of the instrument, and at S is placed a bichromate plate.

The apparatus can only be used in conjunction with a sodium

flame, and a suitable sodium lamp is supplied with it. This consists of a burner supplied with a small piece of platinum wire, on which some pulverised chloride of sodium is placed and made intensely incandescent by means of the non-luminous flame from the burner; the apparatus is pointed to the most intense part of the yellow flame, which can be done easily by means of an adjuster with which the lamp is provided.

The lamp is placed a few inches away from the end of the telescope, and observations are taken in a darkened room or cupboard.

The observation tubes (fig. 50) are made of glass, their ends being ground perfectly true. A brass cap attached to each end enables the tubes to be closed water-tight with small circular glass plates, which are retained in position by the screw-caps. The length of the tubes usually employed are 1 and 2 decimetres (100 and 200 millimetres); an extra 2-decimetre tube, jacketed for invert-sugar determinations, being very useful.

FIG. 50.

If the liquid to be examined is dark in colour, a decimetre (100 mm.) tube is used; whilst if sufficiently pale a 2-decimetre (200 mm.) tube is employed by preference, since the longer the tube the larger the reading and the smaller the chance of error. It becomes necessary, however, in some instances to decolorise a solution before it is possible to take a reading. Decolorisation is usually effected by either passing the solution repeatedly through animal charcoal or by adding basic lead acetate solution and then filtering: examples are given hereafter.

In using the instrument, the lamp is lighted and the flame adjusted to position. On looking through the eye-piece a circle of light is seen divided into two halves by a vertical line, and both halves of the field of view should be exactly equally illuminated. For this purpose the telescope F is, by drawing it out or pushing it together, focussed on the Laurent plate. If the graduated dial is now turned round 3 or 4 degrees to the right of the zero line it will be seen that one half of the field of view will become lighter, the other half darker. The same will be observed on turning it round to the left. That position, however, exactly between these, when both halves of the field of view are equally illuminated, is the zero position of the instrument, and the

latter is first so adjusted that the zero line of the circle coincides with the zero line on the vernier. The half-shadow can now be made lighter or darker, according as the polariser is turned more or less to the right or left of the zero line, by means of the pointer reaching beyond the dial-segment. When the pointer is in the zero position the Laurent's plate and the polarising Nicol have the same wave direction, and consequently, if at the same time the analyser is placed in the zero position, both halves of the field of view appear black. The nearer the pointer is to the zero line, the darker the half-shadow will be, and the more sensitive the apparatus; but when the solution being examined is not quite transparent, the pointer must be moved more or less away from the zero line, so that the field of view is clear. For the majority of experiments the position of the pointer at $7\frac{1}{2}°$ is most suitable; the apparatus is therefore usually so adjusted that in this position the dial and vernier read exactly 0. When the pointer is moved, of course, the zero point of the apparatus changes and no longer corresponds with the zero line of the dial. The difference between the latter and the zero position of the apparatus must either be taken into account—this is the most simple way—or else, after the graduated dial has been moved to 0, the apparatus must be again placed in the zero position; to do this, the analysing Nicol is turned by means of the screw T so far to the right or left until both half-shadows are equal.

Having adjusted the instrument, if we now take a 2-decimetre (200 mm.) observation tube, fill it with a 10 per cent. solution of cane-sugar and place it in the instrument, it will be found on examination that both halves of the field of view are unequally illuminated. On turning the index by means of the milled-head in the same direction as that in which the hands of a clock move (+), a position will be reached when both halves of the field are equally illuminated. When this position is reached, the reading of the scale indicates exactly the amount of twisting or rotation which the polarised beam of light has undergone in passing through a layer of 10 per cent. cane-sugar solution 2 decimetres (200 mm.) thick; and from this can be deduced the specific rotatory power or opticity of the solution as before mentioned.

It often happens, especially during the time when the apparatus is first taken in use, that a circumstance is mistaken for the zero position of the apparatus which arises when the circle has been turned too far and has gone beyond the sensitive range. In this case the light is also to a certain extent equal, but will hardly

change if the circle is turned round 10, 15 or even more degrees. Special attention must, therefore, be given to this circumstance, especially after the fluid has been filled into the tube and the latter placed in the apparatus, that a sudden change of light and dark is not to be observed in both halves of the field of view when, as described, the circle is turned a few degrees either to the right or to the left of the zero line. When the tube has been placed in the apparatus, the first thing to do is to again adjust

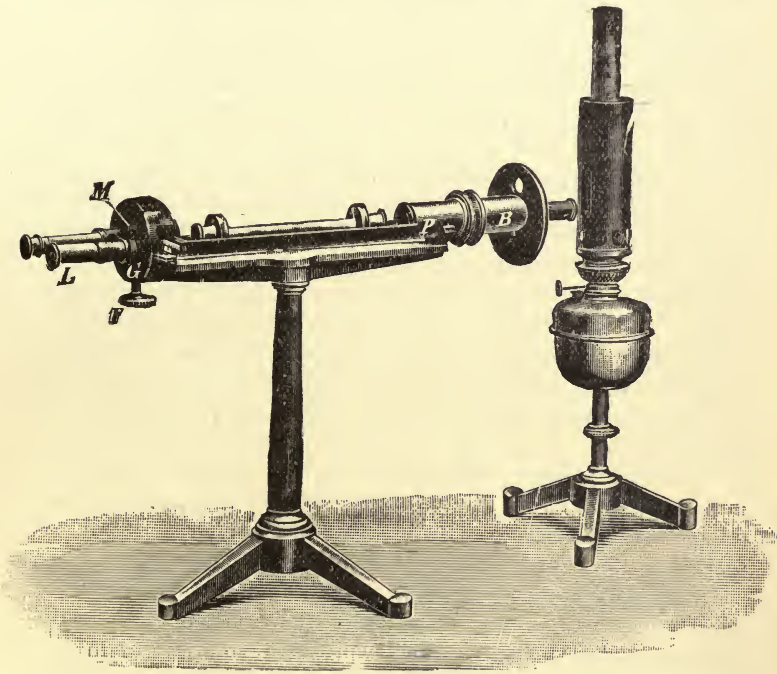


FIG. 51.

the telescope exactly, so that the field of view is again clear and divided into two halves by the vertical line; then the circle is turned round until the half-shadows on both halves are the same, and the result is then read off the scale on the circle.

The graduated dial is graduated right round, that is, into 360 degrees, and, by means of the vernier attached to the index, can be read to the sixtieth of a degree.

The Lippich polarimeter shown (fig. 51) is somewhat similar in construction to the Laurent just described, excepting that a bichromate solution is employed instead of the bichromate glass.

With the Schmidt and Haensch Half-shadow Polarimeter.—The scale is entirely different from that of the Laurent. As the instrument was constructed specially for the use of sugar manufacturers and refiners, its scale is constructed so as to show percentages of cane-sugar directly. It has consequently 100 divisions, which by means of the vernier can be read to tenths of a division. When the 2-decimetre tube is filled with a solution, 100 c.c. of which contains 26·048 grams of pure cane-sugar, the instrument indicates 100 scale divisions. If an impure sugar is employed for making the solution, and with this the reading is 75·8 divisions, then such a sample contains only 75·8 per cent. of cane-sugar. Owing to this difference in the scales of the two instruments, it is necessary, in order to convert half-shadow degrees into Laurent degrees, to multiply by a factor. The divisions of the scale of the instrument bear a fixed relation, however, to the degrees of a sodium-light or Laurent polarimeter. Each single division is equal to 0·3459 of a degree of the Laurent instrument for the ordinary carbohydrates, *excepting cane-sugar*, for which each division of the scale is equal to 0·3469 of a degree. When therefore the half-shadow instrument is employed to determine the specific rotation of a carbohydrate, multiply by 0·3459 for all carbohydrates other than cane-sugar, in order to convert the observed scale divisions into angular degrees for sodium light; in the case of cane-sugar multiply by 0·3469. In order to convert divisions of the half-shadow instrument into degrees $[\alpha]_j$, multiply by 0·3843 for all carbohydrates other than cane-sugar; in the case of cane-sugar, multiply by 0·3854.

Lastly, the phenomenon of "mutarotation" or "bi-rotation" must be mentioned; that is to say, the varying opticity of certain solutions. For instance, freshly prepared solutions of invert-sugar or maltose show abnormal rotation; in the former solution the rotation being above, in the latter below, normal. In either case the solution may be immediately rendered normal by either the addition of a very small amount of alkali (0·1 per cent.) or by boiling. Care must be taken not to overlook this point when analysing worts or freshly prepared solutions containing maltose, dextrose, or levulose.[1] Observations should also always be made with the solution to be examined at a definite temperature, 68° F. (20° C.) being most usually employed.

[1] For recent views concerning mutarotation, see H. E. Armstrong, "Studies on Enzyme Action," *Jnl. Chem. Soc.*, 1903, lxxxiii., 1310.

Specific Rotatory Power or Opticity.

By the above terms is meant the angle indicated by the polarimeter when a layer of the substance, 1 decimetre (100 mm.) in thickness, is examined in that instrument. For instance, when a decimetre tube is filled with turpentine and examined on the Laurent polarimeter, the reading is 22·4; this number, divided by the specific gravity of the turpentine, gives directly the specific rotatory power of that particular turpentine.

The specific rotatory power of an optically active substance in solution is defined as the angle through which a plane polarised ray of light of definite refrangibility is rotated by a column of liquid, 1 decimetre in length, containing 1 gram of the substance in 1 c.c.

It is obvious that a 100 per cent. solution thus indicated could not be used, and we therefore employ a 10, 20, or other per cent. solution, and calculate to 100 per cent. If, for instance, a 10 per cent. solution of pure cane-sugar be examined in a 1-decimetre tube, the angle observed will be 6·66°, and the multiplication of this figure by 10 will give the opticity of cane-sugar = 66·6°.

If instead of a 10 per cent. solution, one of 20 per cent. is employed, the angle observed will be twice as large, and will, consequently, have to be halved before being multiplied by 10.

Should the 2-decimetre (200 mm.) tube be employed, then the reading will have to be halved and multiplied by 5. The opticity is obtained in any case by the following equation:

$$[a]_D = \frac{R}{L \times \frac{G}{100}}$$

in which R is the reading of the polarimeter, L the length of the tube employed in decimetres, and G the number of grams of substance contained in every 100 c.c. of the solution.

For instance, suppose we have a solution containing in every 100 c.c. 13·5 grams of a body, the opticity of which we wish to determine, and upon examining in the 2-decimetre tube we obtain a reading of $+42{\cdot}5°$, then

$$[a]_D = \frac{+42{\cdot}5}{2 \times \frac{13{\cdot}5}{100}} = +157{\cdot}4°$$

The opticity or specific rotatory power of such a solution is therefore $[a]_D = +157{\cdot}4°$.

When the specific rotatory power of a solution is known, the quantity of that substance in solution is readily determined with the polarimeter by the aid of the following formula:—

$$\frac{G}{100} = \frac{R}{L \times [\alpha]_D}$$

G is the number of grams of the substance in 100 c.c. of solution, R the observed angle, and $[\alpha]_D$ the opticity of the substance, L being the length of the observation tube in decimetres.

For example, a solution of cane-sugar of unknown strength is found to give a reading of $+18{\cdot}20°$, then

$$\frac{G}{100} = \frac{18{\cdot}20}{2 \times 66{\cdot}6} \text{ and } C = 13{\cdot}66 \text{ grams.}$$

Every 100 c.c. of the solution, therefore, contains 13·66 grams of cane-sugar.

Again, supposing we have a solution containing maltose and dextrin, and we desire to ascertain the amount of each of these bodies present in the solution. Knowing as we do the opticity of maltose and of dextrin, we proceed thus:—

The solution is found to contain, say, 15·5 grams per 100 c.c. of the mixture of maltose (opticity $[\alpha]_D + 138$), and dextrin ($[\alpha]_D + 198{\cdot}9$). On examining the solution in a 2-decimetre tube, the angle observed is $+52{\cdot}90$. Then

$$\frac{52{\cdot}90}{2 \times \frac{15{\cdot}5}{100}} = [\alpha]_D + 170{\cdot}6.$$

From this number, which is the opticity of the mixed substances, we have to subtract the opticity of that which has the less rotatory power—in this case it will be maltose 138—and divide the result by the difference of the rotatory powers of the two bodies, which here is 60·9 (198·9 − 138).

This will be $170{\cdot}6 - 138 = 32{\cdot}6$, and $\frac{32{\cdot}6}{60{\cdot}9} = 0{\cdot}53$ gram dextrin.

Each gram of substance in solution consists therefore of 0·53 gram of dextrin and 0·47 gram of maltose; consequently the 15·5 grams contain 8·215 grams of dextrin and 7·285 grams of maltose.

Lastly, to convert degrees $[\alpha]_D$ into $[\alpha]_j$, multiply by 1·111 or simply add one-ninth.

To convert degrees $[\alpha]_j$ into $[\alpha]_D$, multiply by ·9 or simply deduct one-tenth.

The following are the specific rotatory powers of the carbohydrates according to Brown, Morris, and Millar.[1]

TABLE SHOWING THE SPECIFIC ROTOTARY POWERS OF THE CARBOHYDRATES.

Substance.	Formula.	Specific rotatory power (absolute).		Specific rotatory power reduced to the common divisor 3·86.	
		$[\alpha]_j$	$[\alpha]_D$	$[\alpha]_{j3\cdot86}$	$[\alpha]_{D3\cdot86}$
Dextrin . .	$(C_{12}H_{20}O_{10})n$	+221	+198·9	+216	+194·4
Cane-sugar .	$C_{12}H_{22}O_{11}$	+ 73·8	+ 66·6	+ 74	+ 66·8
Maltose . .	,,	+153·3	+138	+151	+135·9
Lactose (anhyd.)	,,	+ 61·6	+ 55·4	+ 59·6	+ 53·6
Lactose (cryst.) .	$C_{12}H_{22}O_{11}H_2O$	+ 58·5	+ 52·6	+ 56·6	+ 50·9
Dextrose . .	$C_6H_{12}O_6$	+ 57	+ 51·3	+ 57·4	+ 51·7
Levulose . .	,,	−106 at 15·5° C.	− 95·4	−104·1 at 15·5° C.	− 93·7
Invert-sugar .	$C_6H_{12}O_6 + C_6H_{12}O_6$	− 24·5 at 15·5° C.	− 22	− 24·4 at 15·5° C.	− 21·9

In this table it will be noticed that the absolute specific rotatory power is given, as well as that reduced to the common factor 3·86.

SOLUTION WEIGHT AND SOLUTION FACTORS.

In the analysis of the carbohydrates it is necessary in most instances to know the amount of solid matter present in solution. This may be estimated by evaporating a known bulk to dryness and weighing the residue. Such method, however, is by no means satisfactory, since it becomes essential, in order to remove the last traces of moisture, to continue heating for a considerable time, and by thus heating, the organic substance is to an extent decomposed. On this account it was found preferable to ascertain the amount of matter in solution by taking the specific gravity and dividing the excess weight over water (1000) by a factor.

The original factor employed was 3·85, which was devised by O'Sullivan in 1876,[2] who based it on the assumption that 10 grams of pure maltose or pure dextrin, when dissolved in so much water that the solution measures exactly 100 c.c. at a temperature of 60° F. (15·5° C.), the specific gravity of the solution (10 per cent.) is 1038·5, water being taken as 1000.

[1] *Jnl. Chem. Soc.*, Jan. 1897, 86.
[2] *Ibid.*, 1876, 129.

Assuming that the strength of such solutions was strictly proportional to their specific gravity, a 1 per cent. solution would have a specific gravity of 1003·85, and solutions containing intermediate quantities would have gravities expressed by intermediate values. Consequently, if each per cent. of either of these bodies raised the specific gravity of a solution by 3·85, it would be a simple matter to ascertain the amount present in solution; it would, in fact, be only necessary to subtract 1000 from the specific gravity of the solution and divide the figure so obtained by 3·85.

Thus 100 c.c. of a solution of maltose of a specific gravity of 1055° would contain

$$\frac{1055-1000}{3{\cdot}85}=14{\cdot}285 \text{ grams of that substance.}$$

Later on Brown and Heron[1] came to the conclusion that 3·85 was too low for maltose, the correct divisor being 3·9314, and O'Sullivan afterwards gave the fresh divisor 3·95 for starch conversion products. It was afterwards found that with solutions of the various carbohydrates the specific gravity of the solution was not strictly proportional to the amount of the substance contained in solution, and Brown and Heron[2] proposed the use of 3·86 in all cases as a solution divisor; but this is only correct for a 10 per cent. solution of cane-sugar, which has a specific gravity of 1038·6 at 60° F. (15·5° C.).

Brown, Morris, and Millar[3] have made fresh determinations of the solution weights of a number of the sugars, and of various starch transformation products; the last traces of moisture being removed from the substances dealt with by a process devised by Lobry de Bruyn and Van Leent. It consists in placing the sugar or other body in a small flask, which is placed in a water or oil bath, and connected with another small flask containing anhydrous phosphoric acid, a vacuum being maintained in the apparatus during the drying process. From the results thus obtained, the solution factor was determined for various concentrations. They were found, as in the case of cane-sugar, not to be directly proportional to the percentages present in solution, but might be expressed in the form of a series of curves. These are given in a table, and by consulting this the proper solution factor for any concentration of any of the sugars given can be found by inspection.

The solution factors for solutions of several of the sugars and starch transformation products at a density of 1055° and at a

[1] *Jnl. Chem. Soc.*, 1897, 618. [2] *Ibid.*, 602. [3] *Ibid.*, 72.

temperature of 60° F. (15·5° C.) are shown, Plate 1. fig. 52, but the original paper referred to should be consulted where extreme accuracy is essential.

Brown, Morris, and Millar point out, in defence of their having used the 3·86 factor for starch conversion products, that, so far as ascertaining the percentage of the constituents is concerned, the factor employed is a matter of indifference, provided the specific rotatory powers and cupric reducing values of the constituents corresponding with those of the particular divisor taken are used in the calculation. They can be readily calculated into the true amounts as soon as the true factor for the particular starch conversion is known. At the same time, attention is called to the fact that this is only strictly correct when the solutions are of approximately the same density, and the constituents possess identically the same divisor; but they do not consider that the error thus introduced is sufficiently large to vitiate their former work, or the conclusions based on it.

When the curves of the divisors for the different grades of starch conversions are examined, it is found that for equal concentrations the divisor for high conversions is greater than that for low, in fact there appears to be some inverse ratio between it and the amount of apparent maltose present.

It was found that if the mixed products of starch conversions were assumed to consist of dextrin and maltose, and that the maltose, which, according to the amylöin theory, exists in combination with dextrin, was assumed to have the same solution density as free maltose, it became possible to obtain by calculation the divisor for the amylöin or dextrin constituent. This was done for various conversions, and the curve thus found is given in the table.

Though, as stated, it is somewhat improbable that the solution factor for combined and free maltose would be the same, yet it was found that when this dextrin curve was used in conjunction with the maltose curve it was possible to determine, within certain limits of concentration, and with a fair amount of accuracy, the solution divisor for the mixed products of any starch conversion brought about by diastase, the apparent maltose percentage being either obtained from the opticity or cupric reducing power of the solution.

We thus understand that where carbohydrate bodies have to be examined for cupric reducing power, their amount is most frequently deduced from the specific gravity of their solutions by means of a solution factor. Consequently, it is usual to affix

in small characters the solution factor which has been used in any particular case, such as $K_{3\cdot 86}$ or $K_{3\cdot 85}$. This means that the amount of solid matter in the first instance was estimated by the 3·86 factor, in the second by the 3·85.

The absolute reducing power may be readily obtained from the statement of these on the 3·86 factor when the true solution factor is known. Thus the reducing power of maltose is $K_{3\cdot 86} = 61$. If, as O'Sullivan assumed, the true solution factor for maltose is 3·9314, then its real reducing power is

$$3{\cdot}86 : 3{\cdot}9314 :: 61 : 62{\cdot}12.$$

Cupric Oxide Reducing Power.

It is shown in subsequent pages that several of the carbohydrates reduce Fehling's solution, or, in other words, precipitate different amounts of the copper contained in that standard solution. According to the amount of copper precipitated, so is the power of the carbohydrate in solution. The cupric oxide reducing power is based upon the specific cupric reducing power of a substance referred to dextrose as a standard of 100, and such a figure is indicated by the letter K. Thus $K = 50$ signifies a solution having half the reducing power of dextrose. As the amount of reducing carbohydrate is almost invariably determined by means of a solution factor previously referred to, it is convenient to add the divisor which has been used: thus $K_{3\cdot 86} = 50$ explains that the reducing power is expressed on solid matter determined by the factor 3·86.

Brown, Morris, and Millar propose to refer the reducing power of carbohydrates to maltose taken as 100, and for indicating this they use the letter R; thus $R_{3\cdot 86} = 50$ indicates that the substance has a reducing power of half that possessed by maltose, when the amount of the substance is determined by means of the factor 3·86.

The cupric oxide precipitated by the various carbohydrates, under favourable conditions, is as follows:—

1 gram CuO = ·7435 maltose.
·4535 dextrose.
·4715 invert-sugar.

Solution Factors for Carbo-hydrates at Various Densities. Specific Gravity at 15·5° C. (From Brown, Morris and Millar, *Journal of the Chemical Society,* 1897, vol. lxxi., p. 72.)

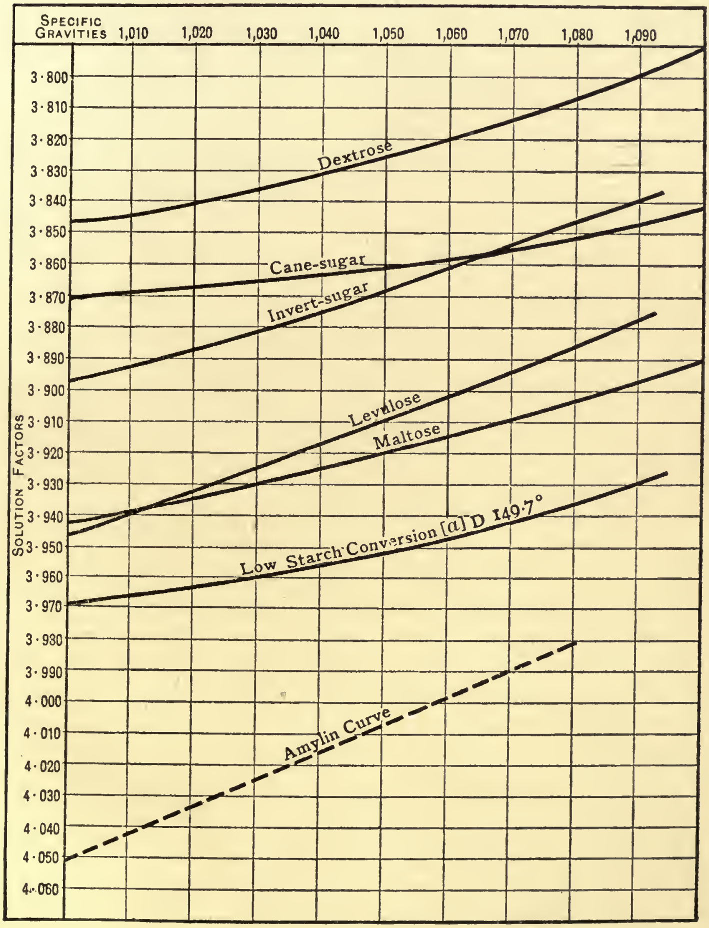


FIG. 52.

[*To face p.* 60.

PART III.

CARBOHYDRATES AND ALLIED SUBSTANCES. PROTEIDS OR ALBUMINOIDS AND ENZYMES.

Carbohydrates and Allied Substances.

The name *carbohydrate*, embracing a very large number of organic bodies, was originally applied to compounds which contain in the molecule six atoms of carbon, or a multiple of this number, together with hydrogen and oxygen present in the proportion in which these elements unite to form water. This water, expressed by the formula OH_2, contains two atoms of hydrogen and one atom of oxygen to form the molecule. In cane-sugar, expressed by the formula $C_{12}H_{22}O_{11}$, it will be seen that the same ratio between the hydrogen and oxygen atoms exists, viz. 2 : 1. In inorganic compounds the term "hydrate" is applied to those substances which actually contain hydrogen and oxygen combined as water: thus calcic hydrate $Ca(OH)_2$ when gently heated gives off the combined water $Ca(OH)_2 = CaO + OH_2$. Hence it was that the carbohydrates were designated as hydrates of carbon. The term carbohydrate, however, applied to the group of compounds about to be considered, is in this sense a misnomer, since the elements of hydrogen and oxygen are not contained within the molecule of these compounds in the same sense as would be understood in the case of an inorganic substance. The term was deduced from a consideration of the empirical formulæ of these bodies before their constitution was as well understood as at the present time.

Recent researches, especially those by Emil Fischer, have shown that the carbohydrates possess an exceedingly complicated molecular structure, and that the group term carbohydrate includes a very large number of substances which may be subdivided into groups having relationships and properties analogous

to, and not greatly differing from, the elementary group of the hydrocarbon derivatives.

Most of the carbohydrate compounds occur in the vegetable organism, but some few are also found in the animal kingdom. The larger number are well known as constituting articles of food, and it is needless to say that the majority are of the highest physiological importance. They consist of several isomeric groups,[1] most of whose members when in solution exhibit active optical properties deviating the plane of polarised light either to the right (+) or to the left (−), and are therefore said to be optically active.

The more important carbohydrates which have an importance from the brewing point of view are, according to their empirical composition, divided into the following groups:—

A. THE PENTOSE GROUP ($C_5H_{10}O_5$).

(*a*) **Aribinose.**
(*b*) **Galactoxylan.**
(*c*) **Xylose, Amylan,** and **Furfural.**

B. THE CELLULOSE GROUP ($(C_6H_{10}O_5)_n$).

(*a*) **Celluloses** including the **Hemicelluloses** and **Oxycelluloses.**

The molecular weight and constitution of most of the members of this group are unknown, or have only been ascertained with approximate accuracy.

(*b*) **Starch.** *Amylo-cellulose.* *Granulose.* *Soluble Starch.*
(*c*) **Dextrins.** *Amylo-dextrins.* *Erythro-dextrins.* *Achroo-dextrins.*
(*d*) **Inulin.**

C. THE HEXOSE GROUP $C_6H_{12}O_6$.

(*a*)	**Dextrose.**	(*d-glucose*)	*Aldose.*
(*b*)		*Galactose*	
(*c*)		*Mannose*	
(*d*)		*Levulose*	*Ketose.*

[1] **Isomeric bodies** or **isomers** are bodies of identical empirical composition, but vary in properties. Thus starch, dextrin, cellulose, etc. have all the same percentages of carbon, hydrogen, and oxygen, though different in many other respects.

D. THE DISACCHARIDE GROUP ($C_{12}H_{22}O_{11}$).

(*a*) **Cane Sugar** or **Saccharose.**
(*b*) **Maltose** or **Malt Sugar.**
(*c*) **Lactose** or **Milk Sugar.**

E. THE POLYSACCHARIDES.

(*a*) **Malto-Dextrins.** $(C_{12}H_{22}O_{11})_n$.
$(C_{12}H_{20}O_{10})_n$.
(*b*) **Raffinose.** $C_{18}H_{32}O_{16}$.

We need only discuss the characteristics of the more important of these bodies, thus:—

A. THE PENTOSE GROUP ($C_5H_{10}O_5$).

Aribonose. Galactoxylan. Xylose. Amylan and Furfural.

The pentoses and their derivatives are found as constituents of the husk and gummy matters of barley and malt. The pentoses yield furfural when hydrolysed [1] by acids, and to this compound is due the objectionable smell which always accompanies any attempt to the further conversion of "spent" grains by means of acid. Furfural is also formed to a slight extent at the mashing stage, the acidity of the mash inverting galactoxylan and then xylose into this compound.

B. THE CELLULOSE GROUP $(C_6H_{10}O_5)_n$.

Hemicelluloses.
Oxycelluloses.
Starch. *Amylo-cellulose, Granulose, Soluble Starch.*
Dextrins. *Amylo-dextrins, Erythro-dextrins, Achroo-dextrins.*
Inulin.

CELLULOSE, HEMICELLULOSE, OXYCELLULOSE $(C_6H_{10}O_5)_n$.

The celluloses constitute the fundamental material of the structure of all plants from the highest tree to the lowest fungus. They are built up from soluble carbohydrates contained in

[1] **Hydrolysis.**—The term was suggested by Dr Armstrong. It is the term used where the assimilation of the elements of water by a molecule of any substance is immediately followed by its splitting up into other compounds.

Decompositions, like that of starch into dextrose, of cane-sugar into dextrose and levulose, of the fats into glycerine and an acid, or of ordinary ether into ethylic alcohol, which involve the fixation of the elements of water, may all be said to be the result of hydrolysis.

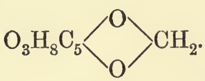
protoplasm, and, with the exception of the very young cellular tissue, always contain mineral matter. Cellulose is therefore very seldom found pure, inasmuch as other substances pass from the cells into the membrane, and there become fixed. If we microscopically examine a thin section of wood or a little pith of the elder, we see that the mass is made up of a great number of irregularly shaped cells, and these are composed of cellulose. Whilst the wood or other vegetable growth is still young the substance of the cells is almost pure, but as the structure gets older the cells become encrusted with resinous and other secretions, and frequently assume a yellow, brown, or red tint. The sources of cellulose are practically endless, and the forms in which it appears are very numerous. Cotton is almost pure cellulose, so also is elder pith, whilst we find it in very hard form in the date stone and in vegetable ivory. Barley contains about 7 per cent. of it, cotton 91·35 per cent., straw 46·22 per cent., and in different kinds of wood the percentage varies from 30 to 63. The purest forms of cellulose are cotton-wool, frequently washed linen, and unglazed paper. If either of these substances be washed, first with weak alkali, and, after every trace of the alkali has been removed, by distilled water, then submitted to the action of weak hydrochloric acid, and finally again washed with distilled water, we obtain cellulose uncontaminated by foreign substances. As such, it is insoluble in the ordinary solvents such as water, alcohol, ether, etc., or by prolonged boiling with dilute acids or alkalies, but it dissolves in an ammoniacal solution of cupric oxide, or in a strong solution of zinc chloride and hydrochloric acid.

Cellulose is a white, tasteless, odourless, innutritious substance, which readily takes up moisture, but is unacted on by it, and unaffected by exposure to air. It is coloured brown by iodine solution, and is about one and a half times as heavy as water.

A mixture of strong nitric and sulphuric acids converts cellulose into nitro-cellulose or gun-cotton, $C_6H_7(NO_2)_3O_5$, a substitution which is reversed by alcoholic solution of potassic hydrosulphide, reproducing cellulose. Besides ordinary forms of cellulose, which in general resist the action of acids, alkalies, and enzymes, there are certain celluloses which undergo hydrolysis with comparative facility. Instances of these are the *hemi-celluloses*, which in the seeds of some plants constitute the reserve material stored by the parent plant for the sustenance of a future plant whilst in embryo. Such reserve material takes the place of the starch stored in the endosperm of the barleycorn. This cellulose is

readily hydrolysed by acids, and is converted into soluble saccharine matters by the agency of enzymes secreted by the growing plant. As examples, mention may be made of the cellulose forming the walls of the starch containing cells of the endosperm of barley, the dissolution of which takes place during germination by the enzyme cytase, and the amylo-cellulose of ordinary starch granules which is slowly hydrolysed by diastase in the cold, but with facility at a temperature of 140° F. (60° C.).

Cross and Bevan have shown that there are still further modifications of cellulose present in many vegetable tissues, such as those of the barley plant; and these, since they contain larger percentages of oxygen than ordinary cellulose, have been named "oxy-celluloses." They are extremely resistant to the action of dilute alkalies, and, like the pentose sugars, the oxy-celluloses, when distilled with hydrochloric acid, yield furfural; and upon hydrolysis with dilute acids (1 per cent. sulphuric) are partially converted into a fermentable sugar, having probably the following constitution,



It was whilst studying this class of bodies that the author conceived and patented the idea of treating "spent" grains for the extraction of sugar.[1]

The process consisted in converting "spent" grains in a similar fashion to that in which raw grain is sometimes converted.

"Spent" grains contain from 2 to 10 per cent. of starch; and it was not only to recover this residual starch, but also to act upon the cellulose and convert it also into sugar, that constituted the idea. The sugar obtained was found to be of the furfuroid character, and amenable, to an extent, to the fermentative action of yeast. The results of the author's experiments in conjunction with those of Cross and Bevan have long been recorded;[2] suffice it to say that no commercial progress was made, after lengthy experiments and heavy expenditure.

+ STARCH $(C_6H_{10}O_5)_n$.

Amylo-Cellulose. Granulose. Soluble Starch.

The formula for starch is as shown above, the value of n not being known. Brown and Morris, as is shown hereafter when

[1] Bailey and Ford Patent, No. 1788, 1896.

[2] *Jnl. Fed. Inst. Brewing*, 1897, 75.

considering the malto-dextrins, are of opinion that the formula for starch cannot be less than $5(C_{12}H_{20}O_{10})_{20}$, but it is yet doubtful whether this formula will not at some future date be altered. At any rate we may look upon starch as a carbohydrate containing C, H, and O in the proportions in which these elements unite to form water, and await developments for more light upon the actual multiple of these elements.

Starch is a substance found in the form of small granules in the cells of grain, legumes, and potatoes. It exists in nearly all plants with the exception of the fungi. It is, in fact, the most abundant material to be found in the vegetable world. It is originally formed in the cells of the leaves of plants from the carbon dioxide of the atmosphere, by the agency of chlorophyll (the green colouring matter of leaves) in the presence of sunlight. The starch thus formed may be looked upon as migratory, since by enzymic action it is transformed into soluble sugars, passing into the sap of the plant, and thence carried throughout the plant, building up its fabric, reappearing at different stages of the plant growth as cellulose, gummy matter, lignin, or transformed again into starch as the reserve material in the seeds of cereals, the tubers of the potato, etc. The study of the metamorphoses of starch in the living plant, from the assimilation of carbon dioxide in the green leaves to the formation of starch in the barleycorn, is an interesting study, and yields much valuable information upon the process of germination. The classical researches of Brown and Morris upon the "Physiology of Foliage Leaves"[1] and the "Germination of the Gramineæ,"[2] should therefore be consulted.

Starch is generally obtained in Europe from wheat, maize, potatoes, and rice, and in tropical countries from the stems of the palm and from the tubers of various plants; thus, in the East Indies and the Philippine Islands, starch is obtained from the pith of the sago-palm (*Sagus lœvis* or *Sagus rhumphii*). This comes into the market under the name of sago (a word meaning bread). Arrowroot is the starch of the *Maranta arundinacea* and *indica*, and a few other tropical plants growing in the West Indies, Brazil, and the Southern States of America. Tapioca is derived from *Yatropha manihot*, this, like sago, being subjected to pressure to give the grains a peculiar form.

[1] "A Contribution to the Chemistry and Physiology of Foliage Leaves" (*Jnl. Chem. Soc. Trans.*, 1893, 604–677).

[2] "Researches on the Germination of some of the Gramineæ" (*Jnl. Chem. Soc. Trans.*, 1890, 458–528).

Starch being heavier than water, its density at a temperature of 67·5° F. (19·7 C.) being 1·505, has the property, when mixed with it, of sinking with proportionate rapidity, and we may observe that the bottom layer is remarkable for great toughness. It is never found in a state of purity, but always occurs mixed with a greater or less amount of nitrogenous matter. In order to purify it, until within recent years the starch was mixed with water and allowed to stand until fermentation set in, this being caused by the action of aërial ferments, the result being that the formation of acetic, butyric, and lactic acids in time split up the nitrogenous matter, and a subsequent washing with water left the starch pure.

There are two objections to this method of preparing starch: firstly, the lengthy time necessarily occupied; and secondly, the objectionable odour evolved from the fermentation, which renders the manufacture a nuisance to the neighbourhood. It became necessary, therefore, to adopt fresh means for purifying it, and this is now performed by dissolving the nitrogenous bodies with caustic soda instead of the previous objectionable method of fermentation. The steep water is first made faintly acid by the addition of a little sulphuric acid, and the starch is afterwards treated with water containing $\frac{1}{350}$th of its weight of caustic soda, or a solution containing 200 grains or $\frac{1}{2}$ ounce of the alkali per gallon.

The external characteristics of starch granules can only be distinguished by the aid of the microscope on account of their minute size; and by the aid of this instrument it was discovered that not only does the size of the granules vary in every species of starch, but that each species exhibits its own particular form and structural markings. Adulteration can thus be detected, and admixture of cheap potato starch with the more expensive wheaten starch may be approximately measured by microscopical observation.

Though the cells of every species of starch vary in size and shape, they nevertheless exhibit certain similarities in structure. Each has a dark point, central in some, eccentric in others, known as the "hilum." Round this are seen a series of concentric lines, an appearance caused by the peculiar structure of the granules, which are built up of layers containing varying amounts of water. The hilum is always rich in water, and each layer alternately contains more or less water, the outside layer being always most free from moisture and richest in substance. As a consequence of the proportion of water increasing from the outside of the granule inwards to the hilum, fissures radiating from the hilum towards the periphery often arise as the granule becomes

dry. By steeping starch granules in alcohol, which entirely deprives them of water, all appearance of stratification disappears, but the lines reappear if the granules are moistened with water. If they are treated with dilute alkali or acid, the appearance of stratification is rendered much more distinct.

The following sketches, figs. 53 to 64, Plates II. to VII., show the microscopical appearance of the more important starches; and the table, by Galt,[1] gives their outline, measurement, surface, hilum, and markings.

Air-dried starch contains from 15 to 20 per cent. of moisture, the last traces of which it retains with remarkable pertinacity, consequently it is almost impossible to remove the whole of the moisture by heat alone without at the same time causing a chemical alteration in the starch substance itself. To avoid this, Dafert[2] proposes to remove the hygroscopic water by drying the starch *in vacuo* at 212° F. (100° C.). Absolutely dry starch attracts moisture with such avidity that when moistened with water a perceptible rise of temperature takes place, a phenomenon which shows that the last portions of water are in a state of chemical combination.

Starch is an exceedingly inert substance, insoluble in cold water, alcohol, ether, and ammonia cuprate, the latter showing its difference from cellulose; but it dissolves in potassic hydrate solution, in consequence of the formation of a potassium derivative.

On treating with acetic anhydride it is converted into a tri-acetate, $C_6H_7O_2(C_2H_3O_2)_3$.

It may be heated, when dry, to a temperature of 300° F. (148·8 C.) without change, but by a temperature of from 300° to 400° F. (148·8° to 204·4° C.) it is converted into a substance soluble in cold water, termed dextrin or British gum, and at temperatures above this it is decomposed.

Starch is largely used in the arts for laundry purposes, paper sizing, bookbinding, weaving and finishing calicoes, also for preparing the thickening for colours and mordants in calico printing, for dusting the formes in metal founding, and a variety of other purposes.

So long ago as 1716 Leuwenhoek asserted that the cell-walls of the granules differ from the cell contents; and Raspail confirmed this assertion, believing, however, that the cell contents were identical with gum arabic. On the other hand, Guibort came to the conclusion that both the above possessed the same chemical

[1] *The Microscopy of the Starches.*

[2] *Jnl. f. prakt. Chem.*, lxxiii. 51.

FIG. 53.—Potato Starch. ×116.
(Galt-Baillière, Tindall, and Cox.)

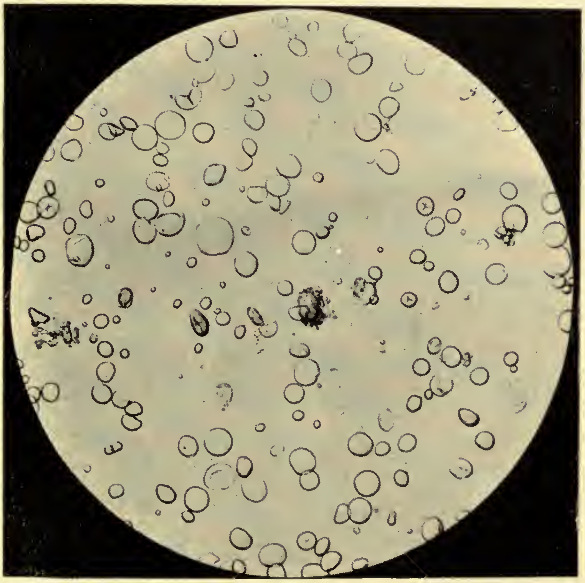


FIG. 54.—Rye Starch. ×116.
(Galt-Baillière, Tindall, and Cox.)

[*To face p* 68.

FIG. 55.—Rice Starch. ×300.
(Macmillan.)

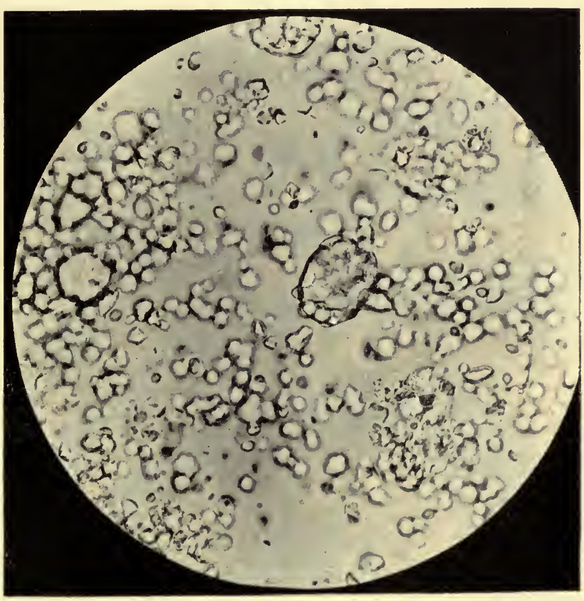


FIG. 56.—Oat Starch. ×380.
(Galt-Baillière, Tindall, and Cox.)

[*To face p.* 68.

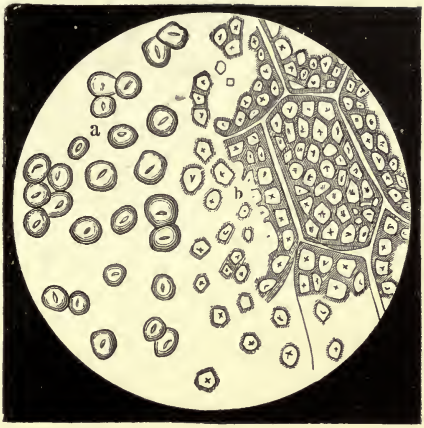


FIG. 57.—Maize Starch. ×300.
(Macmillan.)

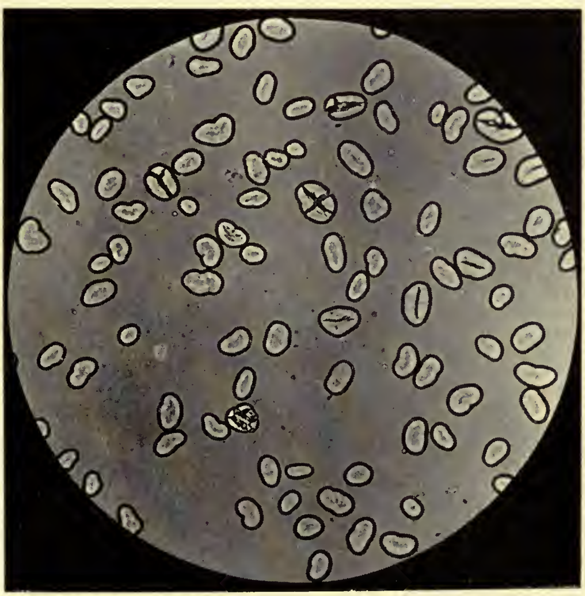


FIG. 58.—Pea Starch. ×116.
(Galt-Baillière, Tindall, and Cox.)

[*To face p.* 68.

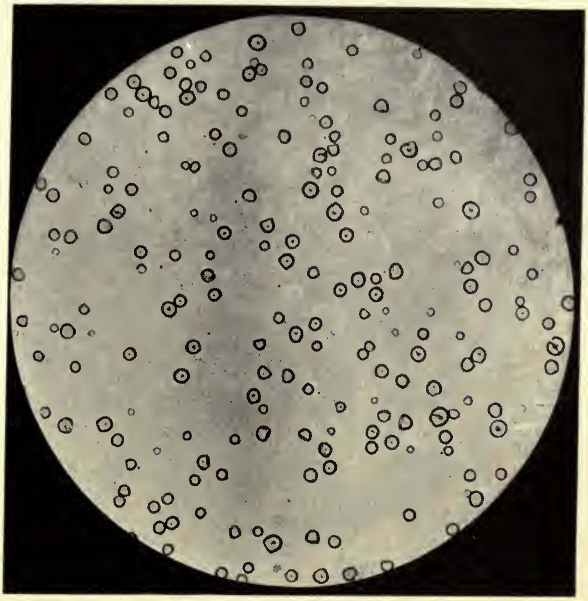


FIG. 59.—Tapioca Starch. ×116.
(Galt-Baillière, Tindall, and Cox.)

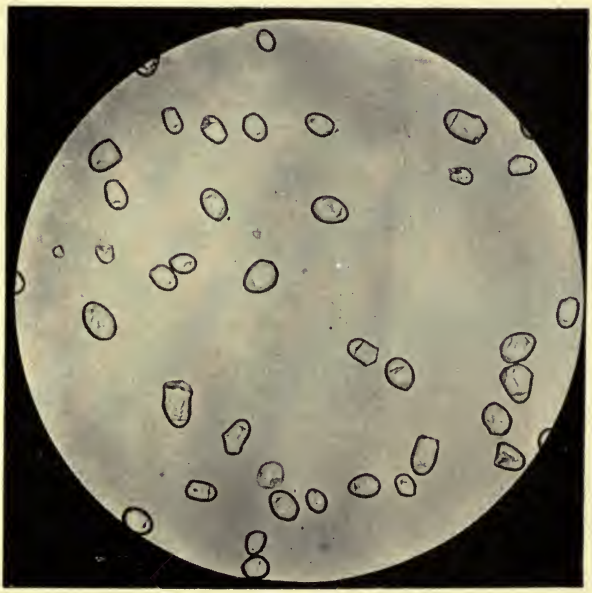


FIG. 60.—Sago Starch. ×116.
(Galt-Baillière, Tindall, and Cox.)

[*To face p.* 68.

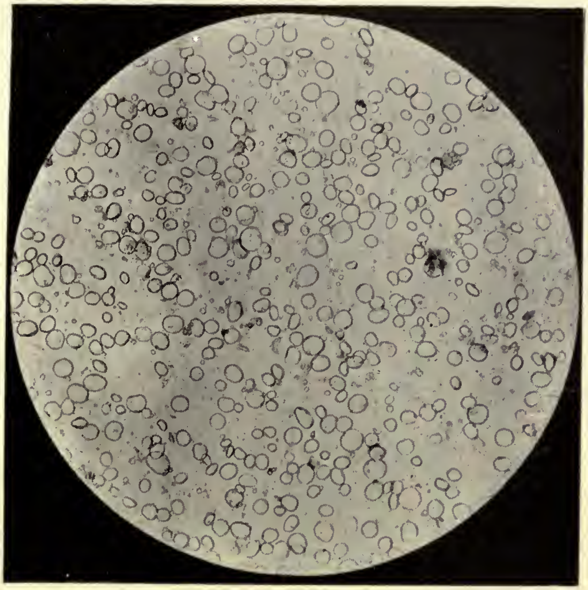


FIG. 61.—Barley Starch. × 116.
(Galt-Baillière, Tindall, and Cox.)

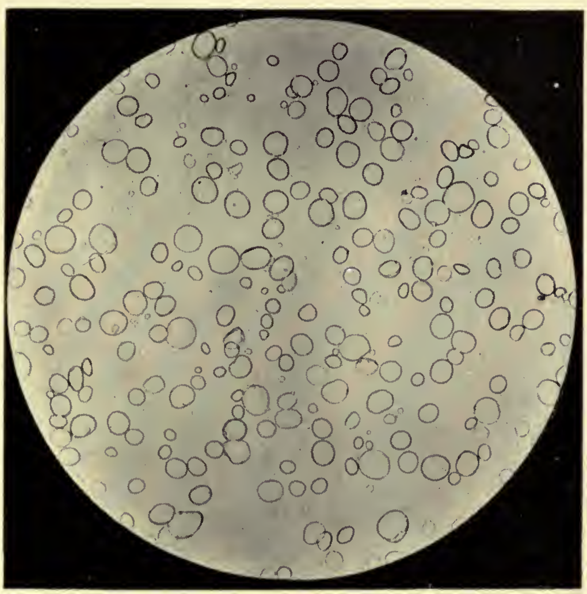


FIG. 62.—Wheat Starch. × 116.
(Galt-Baillière, Tindall, and Cox.)

[*To face p.* 68.

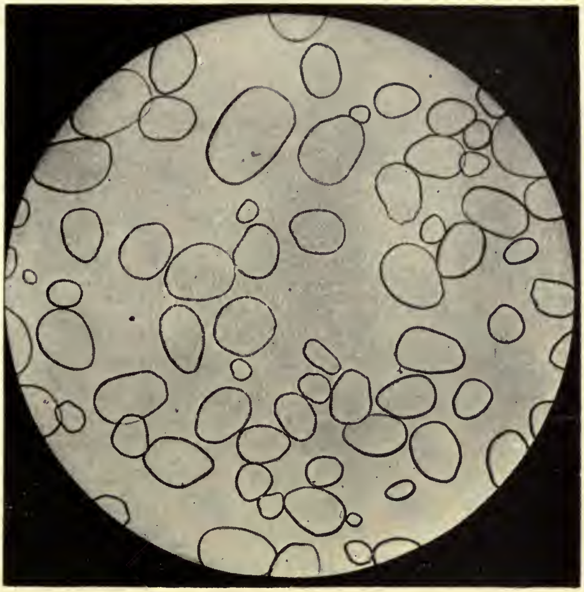


FIG. 63.—Tous les Mois Starch. ×116.
(Galt-Baillière, Tindall, and Cox.)

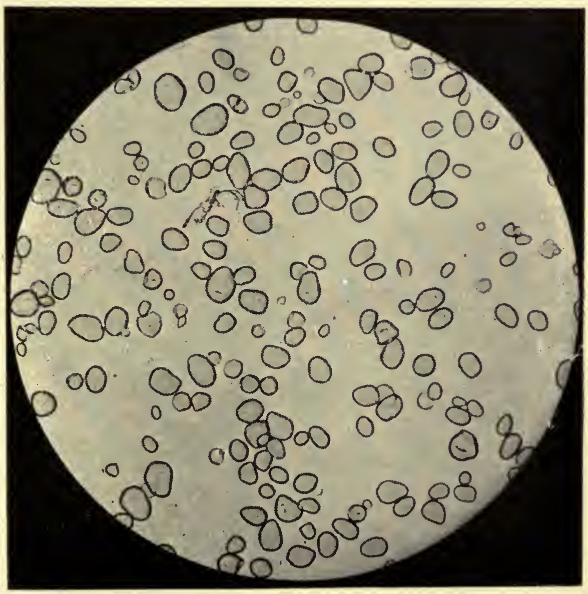


FIG. 64.—Bermuda Arrowroot Starch. ×116.
(Galt-Baillière, Tindall, and Cox.)

[*To face p.* 68.

Table showing the Leading Microscopical Characters of the more important Starches.

Fig.		Outline.	Measurement.	Surface.	Hilum.	Markings.
60	Potato.	Oval or elliptical.	mm. $\frac{1}{25}$ in long diameter; $\frac{1}{37}$ in short diameter.	Uniformly but slightly convex.	Dark spot near narrow end.	Concentric rings; closed, or almost closed, curves.
61	Rye.	Circular.	$\frac{1}{40}$	Convex, spherical.	Crucial or radial, central, large.	Exceedingly faint complete concentric rings.
62	Rice.	Rectilinear and polygonal.	$\frac{1}{256}$	Flat.	None.	None.
63	Oat.	Rectilinear and polygonal.	$\frac{3}{500}$	Flat or slightly convex or concave.	None.	None.
64	Maize.	Rectilinear and polygonal.	$\frac{1}{70}$	Uneven, and slightly concave.	Stellate or irregular, large, central.	None.
65	Pea.	Reniform.	$\frac{1}{28}$ in long diameter; $\frac{1}{40}$ in short diameter.	Convex, with central longitudinal depression.	Large, slit-like, longitudinal, central.	Appearance of concentric rings forming closed curves.
66	Sago.	Rounded; some partly angular.	$\frac{7}{200}$ in long diameter; $\frac{9}{400}$ in short diameter.	Uniformly and highly convex.	Near large and rounded end, large, slit-like or irregular, transverse.	Occasionally a few very faint, generally incomplete, concentric rings.
67	Tapioca.	Rounded; some partly angular.	$\frac{1}{70}$	Uniformly convex.	Slit-like, transverse, stellate, central.	Like sago, but still more indistinct.
68	Wheat.	Circular, or nearly so.	$\frac{1}{50}$	Convex.	Dark spot, eccentric.	Occasionally a few exceedingly faint concentric rings.
69	Barley.	Circular, lenticular.	$\frac{3}{200}$	Convex.	Dark spot, eccentric, seldom apparent.	Very rarely, faint indication of concentric rings.
70	Bermuda arrow-root.	Oval.	$\frac{1}{28}$ in long diameter; $\frac{1}{45}$ in short diameter.	Uniformly but slightly convex.	Near broad end, circular, crucial, transverse line or slit.	Faint concentric rings, in a few cases extending about two-thirds of length of grain.
71	Tous-les-Mois.	Irregularly oval or elliptical.	$\frac{7}{100}$ in long diameter; $\frac{5}{100}$ in short diameter.	Uniformly but very slightly convex.	Dark spot near narrow end.	Concentric rings extending for less than one-third of length of grain.

composition, and that they only differed physically.[1] The structure of the starch granule was then carefully examined by Fritsche,[2] and the classical researches of Nægeli [3] proved that starch is a mixture of several isomeric compounds. Its internal portion consists of what is termed *granulose* with a small portion of starch cellulose, whilst the outer coating consists principally, if not entirely, of this latter substance. The outer coating or cellulose of the starch granule protects the inner content or granulose from being acted upon either by cold water or diastase, and before any action can therefore take place it becomes necessary to burst the granules either by attrition or heat.

There is great divergence of opinion as to whether or not the granulose upon being set free from the granule enters into a state of solution. Some say that it does not, since if it did so it would readily pass through cell membrane, which property it does not possess. Brown and Heron believe that the granulose is in a true state of solution, and that the viscosity of starch paste is due to the swollen state of the cellulose. They found the viscosity to vary considerably. The more slowly the starch has been dried and the lower the heat at which this has been effected, the more viscous the solution; in fact they found the difference in viscosity, due to the varied methods of preparation, to amount to more than three to one. Moritz and Morris express the opinion that on boiling starch the granulose is converted into soluble starch.

In any case, when starch is heated with water the granules undergo a singular change in their structural condition. They take up large quantities of water, become swollen, and in time, if the temperature of the water is high enough, burst. A gelatinous mass is formed which is designated as starch paste, the process itself being termed the production of starch paste, or the gelatinisation of starch.

During the heating the volume of the liquid becomes greater. According to Payen, the increase of volume amounts to 142 per cent. at a temperature of 140° F. (60° C.), and to 1255 per cent. at 158°–161·6° F. (70°–72° C.). The higher the temperature of the water, the greater the swelling of the granules, and also the greater the diffusion. The temperature at which starch granules begin to swell, and also that at which a complete transformation into paste occurs, differs according to the nature of the starch.

Soluble Starch is conveniently prepared, according to

[1] *Jnl. Chem. Med.*, v. 9. [2] *Pogg. Ann.*, xxii. 129.
[3] *Jahresber. d. Chemie*, 1859, 544.

Lintner,[1] by digesting potato starch with a 7·5 per cent. solution of ordinary strong hydrochloric acid at a temperature of 60° F. (15·5° C.) for one week, afterwards washing the starch by repeated decantation until the washings are perfectly free from acid; or, according to Brown and Morris,[2] a 12 per cent. solution of hydrochloric acid may be employed, in which case digestion for twenty-four hours suffices. The starch granules undergo no apparent physical change by the process, as is shown by microscopical examination, in which case it will be seen that the granules retain their original appearance.

Soluble starch is precipitated by a large addition of alcohol, also by many metallic oxides, as lime, baryta, and lead oxide. It is coloured an intense deep blue by iodine solution, this property having been discovered by Gaultier de Clanbry in 1814. The reaction, however, is much influenced by temperature; the lower the temperature the more sensitive is the reaction. At or near the boiling-point of water the coloration does not appear at all, but a deep blue makes its appearance as the solution cools, provided the solution is not subjected to prolonged heating. The blue colour does not show itself so readily, nor is it so pure, in the presence of other bodies such as tannin, malt extract, beer, yeast, etc. Whether the blue substance formed is a true chemical compound or not has not been definitely settled.

The colour also varies according to the nature of the starch; thus potato-starch gives a deep blue, wheat-starch a colour inclined to violet. This is accounted for by a larger amount of the cellulose, which is coloured brown by iodine, being contained in some of the starches.

Two starches have been met with by Dafert and Kreusler,[3] the one in a species of rice, the other in a kind of millet, which are coloured from red to brown by iodine solution. The opticity of soluble starch is given as $[\alpha]_{j\,3\cdot86} + 216°$ by Brown and Morris, and as $[\alpha]_D + 200°$ by Lintner. Brown, Morris, and Miller state that the opticity in 2·5 to 4·5 per cent. solution is at 60° F. (15·5° C.) $[\alpha]_D$ 202°. It has no reducing action on Fehling's solution. When acted on by diastase in the cold it is decomposed in ten minutes to the No. 8 equation. When, however, the action of diastase is allowed to proceed for ten or twelve days in the cold, soluble starch is entirely converted into maltose.[4]

[1] *Jnl. f. prakt. Chem.*, xxxiv. 378.
[2] *Jnl. Chem. Soc.*, 1889, 450.
[3] *Landwirth. Jahrbuch*, xiii. 767, xiv. 831.
[4] Lintner, *Jnl. f. prakt. Chem.*, 1887.

By the action of diastase or acid on soluble starch, under suitable conditions, identical sugars are formed, and the inference naturally arises that the intermediate products (malto-dextrins), formed by the respective agents, are identical.

The transformation of soluble starch to dextrins and malto-dextrins can be followed to a certain extent by the iodine reaction, the deep blue tint produced in soluble starch changing to red upon the starch hydrolysing to dextrin.

Brown and Morris,[1] by employing Raoult's method, found the molecular weight of soluble starch to vary from 20,000 to 30,000; and on the asumption that the stable dextrin of the No. 8 equation constitutes one-fifth of the molecule, they concluded that its molecular weight is 32,400, and its formula $5(C_{12}H_{20}O_{10})_{20}$. The question the brewer has to take into consideration is how best and most economically he can transform starch into dextrin, maltose, and malto-dextrin, in proportions suitable for any particular beer. No matter whether he employs the starch of a cereal such as rice or maize, he obtains the same fermentable products as those produced by the employment of oats or barley. Certain starches, however, produce somewhat objectionable flavoured worts, and such worts are prone to quickly acidify; hence it is that only certain raw grain starches are employed by the brewer.

+ Dextrin $(C_6H_{10}O_5)_n$.

Amylo-dextrins. Erythro-dextrins. Achroo-dextrins.

By heating dry starch to a temperature of from 300°–400° F. (148·8–204·4° C.), as already mentioned, a substance, soluble in cold water, termed dextrin or British gum, is produced. Dextrin forms an intermediate stage in the conversion of starch, whether effected by mineral acids or malt-extract (diastase). There are three kinds of dextrin, designated *amylo*, *erythro*, and *achroo*, the amylo- and erythro-dextrins giving a red or brownish colour with iodine solution, the achroo-dextrins being unaffected by this reagent. There has been great controversy as to the existence of different types of dextrin, Greissmayer, O'Sullivan, Nægeli, and other investigators describing several.

The dextrins are neutral, tasteless substances, soluble in water, dilute alcohol or naphtha, but insoluble in absolute alcohol or ether.

[1] *Jnl. Chem. Soc.*, 1889, 465.

C. The Hexose Group $C_6H_{12}O_6$.

+ *Dextrose (d-glucose). Galactose. Mannose.*
− *Levulose (fructose).*

+ Dextrose (*d-glucose*) $C_6H_{12}O_6$.

Dextrose or *d-glucose* is one of the sugars found in great abundance in the vegetable kindom. Ripe sweet fruits, such as grapes, plums, figs, etc., contain, in addition to levulose and small quantities of cane-sugar, large quantities of this substance. It is also met with in the stems and seeds of cereals and in the flowers of many plants from which bees derive honey; it is also found in honey.

Compounds of dextrose are frequently met with, termed glucosides, which readily split up under the hydrolysing action of dilute acids or enzymes into dextrose and their other constituents. Dextrose is now largely prepared from starch and starchy materials by the action of dilute mineral acids, the product thus obtained consisting of a mixture of dextrin, maltose, and dextrose, the percentages of these sugars depending upon the conditions and length of time the hydrolysis is allowed to proceed. But if the hydrolysis is prolonged to its fullest limit, the dextrin first formed is converted to maltose and the latter to dextrose. In such cases other substances besides dextrose are formed, amongst which is supposed to be a substance termed gallisin. Of their character, little of a satisfactory nature is known.

Dextrose is only half as sweet as cane-sugar; it is readily soluble in water and dilute alcohol, but is completely insoluble in absolute alcohol. It is not charred or blackened by concentrated sulphuric acid, as is cane-sugar, but if heated with solutions of the alkalies a brown coloration is produced, and with dilute acids prolonged heating forms brown substances termed ulmin and ulmic acid. On heating dextrose to temperatures between 230°–309°F. (110°–154° C.) *caramel* is produced. On heating to 340°F. (171° C.) water is given off and glucosan is formed; whilst on still further heating, the substance is decomposed.

Dextrose readily combines with oxygen, and hence is able to reduce the oxides of several metals to the metallic form; whilst in the case of some metals such as copper, the higher oxide is reduced to the lower, viz., CuO to Cu_2O. On this property the methods of determining dextrose quantitatively are founded.

Dilute nitric acid oxidises dextrose to saccharic acid ($C_6H_{10}O_8$).

Solutions of dextrose are readily fermented by yeast, maltose

requiring to be first hydrolysed to dextrose by the enzyme *maltase* contained in yeast, and cane-sugar to be first hydrolysed to invert by the enzyme *invertase* contained in yeast, before fermentation commences and splits the sugar up into alcohol and carbon-dioxide. Upon fermenting, dextrose yields 48·67 per cent. of alcohol. It possesses an optical activity of $[\alpha]_{D\,3\cdot86} = 51\cdot7$, and its cupric-oxide reducing power is $K_{3\cdot86} = 100$.

Dextrose solutions, like those of levulose and maltose, produce an osazone when treated with phenyl-hydrazine.

If 1 gram of dextrose is dissolved in 50 c.c. of water, and 2 grams of phenyl-hydrazine dissolved in 2 grams of 50 per cent. acetic acid are added, the mixture upon being treated throws down a dense yellow precipitate. The action is complete in one hour. The precipitate, microscopically examined, will be found to consist of needle-shaped crystals, some of which may occur in fan-shaped aggregates. The precipitate, when collected, washed with hot water, and dried at 212° F. (100° C.), will be found to be very insoluble in boiling water, which characteristic assists in its identification.

The reaction of phenyl-hydrazine with the hexoses is as follows:—If one molecule of phenyl-hydrazine is allowed to act on one molecule of a hexose, a normal hydrazone is formed:—

$$\underset{\text{Dextrose.}}{CH_2.OH(CH.OH)_4.CHO} + \underset{\text{Phenyl-hydrazine.}}{C_6H_5NHNH_2} = \underset{\text{Hydrazone.}}{CH_2.OH(CH.OH)_4.\underset{\substack{\Vert \\ N-NH.C_6H_5.}}{CH}} + H_2O$$

But if two molecules of phenyl-hydrazine are used, an osazone is obtained:—

$$\underset{\text{Glucosazone.}}{CH_2.OH(CH.OH)_3.\underset{\substack{\Vert \\ N \\ | \\ NH.C_6H_5.}}{C} - CH = N - NH.C_6H_5}$$

Dextrose solutions, like those of some other carbohydrates, also possess the phenomenon of muta-rotation. A freshly-prepared solution shows an opticity nearly twice as great as that given after standing, and the opticity only becomes stationary after a lapse of about 24 hours. Two minutes boiling or the addition of a very small quantity of caustic potash or ammonia (0·1 per cent.), however, at once fixes the rotatory power. Besides by the hydrolysis of starch, dextrose may be prepared from cane-sugar,

in which case the product consists of nearly equal weights of dextrose and levulose, otherwise known as invert-sugar. The solid portion of commercial samples of invert consist of crystallised dextrose, which may be washed with alcohol, dissolved, and recrystallised from methyl-alcohol. The dextrose then separates as hydrate ($C_6H_{12}O_6 + H_2O$).

– LEVULOSE (*Fructose*) $C_6H_{12}O_6$.

Levulose exists in association with dextrose in grapes, ripe fruits, honey, and in the stems and seeds of cereals; and is present in invert-sugar to the extent of nearly half the latter's weight. It may be separated from invert by making a solution, mixing an equal weight of finely-powdered slaked lime, and maintaining for some time at a low temperature. The lime forms a solid compound with the levulose known as insoluble calcic levulosate, while the dextrose forms a compound which is freely soluble, and may be separated by filtration and washing. The residue is then mixed with water, and on passing a current of carbon-dioxide through the liquid, decomposition occurs, the lime being precipitated as carbonate, and the levulose passing into solution. This solution may be rendered anhydrous by evaporation *in vacuo* over sulphuric acid. Thus prepared, levulose is a colourless, uncrystallisable syrup, distinctly sweeter than dextrose, and more soluble than the latter in alcohol. By careful recrystallisation, it may be obtained in fine silky crystals, which melt at a temperature of 203° F. (95° C.).

Levulose turns a polarised ray of light strongly to the left, hence its name, the angle of rotation being $[\alpha]_{D\,3\cdot86} = -93\cdot7$, and it has a cupric-oxide reducing power of $K_{3\cdot86} = 92\cdot4$.

The polarisation is thus more powerful than that of dextrose to the right, so that a solution of invert-sugar, containing equal quantities of each sugar, possesses a distinct levo-rotatory power. The levo-rotatory angle diminishes as the temperature of the solution rises, so that at a certain temperature levulose apparently possesses no rotatory power at all, since the action of the negative levulose is then exactly balanced by the positive rotation of the dextrose, and at about this temperature invert-sugar becomes dextro-rotatory.

Levulose ferments in contact with yeast, but not so readily as does dextrose, a fact which favours the theory that the sluggishness of its fermentation is due to it not being able to diffuse so readily as dextrose through the walls of the yeast cells.

When levulose is heated strongly it is converted into levulosan ($C_6H_{10}O_5$), a body isomeric with glucosan and produced in the same way from dextrose by the expulsion of water. It comports itself in many respects in an almost similar manner to dextrose, and is oxidised into saccharic acid by contact with dilute nitric acid.

Solutions of levulose form an osazone when treated with phenylhydrazine.

D. The Disaccharide Group $C_{12}H_{22}O_{11}$.

Cane-sugar or Saccharose. Maltose or Malt-sugar. Lactose or Milk-sugar.

+ Cane-Sugar $C_{12}H_{22}O_{11}$.

Cane-sugar is freely soluble in water, a cold, saturated solution at 60° F. (15·5° C.) contains about 66·3 per cent., a boiling solution about 82·5 per cent. The gravity of a solution increases in a slightly greater ratio than the percentage of sugar, and the difference in ratio becomes still more pronounced as the quantity is further increased.

Cane-sugar is insoluble in ether, slightly soluble in absolute alcohol, and more so in dilute alcohol, the solubility increasing with the dilution of the alcohol. It has no reducing action on Fehling's solution, melts at a temperature of 320° F. (160° C.), and on cooling solidifies to an amorphous mass, which, after some time, becomes crystalline.

When heated for some time to 338° F. (170° C.) it is split up into dextrose and levulose, but if exposed to a higher temperature, 356°–392° F. (180°–200° C.), the mass becomes first yellow and then brown, forming *caramel.* Heated to a still higher temperature, decomposition takes place with evolution of combustible gases and acid vapours, a light porous mass of carbon being left behind.

If a little concentrated sulphuric acid be added to cane-sugar syrup, an immediate and characteristic action ensues; the sugar is deprived, by the acid, of the elements of water, and its carbon separates as a solid swollen black mass, of much greater bulk than the syrup from which it is derived. If, however, a solution containing not more than 30 per cent. of cane-sugar is heated with dilute acid, the sugar, instead of parting with, takes up the elements of water and is hydrolysed into a mixture of dextrose and levulose, otherwise known as invert-sugar. The same change

is brought about when cane-sugar solution is placed, under suitable conditions, in contact with yeast, *invertase*, the enzyme of *monilia candida*, or malt extract. In the latter cases, however, the action is very slow. The reaction which results is the same whichever agent be employed ; it consists in the assimilation of water by the cane-sugar molecule, followed by its separation into the two different kinds of sugar named, which, though of very different properties, have the same formula.

The following equation expresses the change :—

$$\underset{\text{Cane-sugar.}}{C_{12}H_{22}O_{11}} + \underset{\text{Water.}}{H_2O} = \underbrace{\underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Levulose.}}{C_6H_{12}O_6}}_{\text{Invert-sugar.}}.$$

Yeast ferments cane-sugar solutions of weak or medium strength, but is unable to ferment solutions of 50 to 60 per cent. strength, or any solutions to which large quantities of glycerine have been added : the enzyme *zymase*, however, ferments these solutions readily.

Cane-sugar forms compounds (saccharates) with several bases, such as potassium, strontium, lead, etc., all of which are decomposed by carbon-dioxide.

It does not form a compound with phenyl-hydrazine, but when heated in a solution of the acetate of that base, it first suffers inversion into dextrose and levulose, these latter sugars uniting with the phenyl-hydrazine to form glucosazone. Cane-sugar solutions of medium dilution and under suitable conditions are completely fermentable by yeast, but in such instances the fermentation is not a direct one, the sugar being first split up into invert.

Many bacteria are able to induce peculiar fermentations in solutions of cane-sugar, in which such bodies as lactic acid, butyric acid, mannitol, etc., are produced.

+ Maltose $C_{12}H_{22}O_{11}$.

Although belonging to the same group as cane-sugar, maltose has few properties in common with that most familiar substance. It nowhere in nature exists ready formed to any great extent. It is surmised that plants, during their growth, transform a portion of their starch into maltose as and when they require the latter for food, but the amount required is so infinitesimal that no large quantity is found to exist therein.

Maltose was discovered by De Saussure in 1819, but the discovery was overlooked or forgotten. It was again discovered by Dubrunfant[1] in 1847, but again overlooked or forgotten. In 1872, however, C. O'Sullivan[2] rediscovered it.

O'Sullivan prepared it by acting upon gelatinised potato starch, with diastase at about 90° F. (32·2° C.). Chloroform was added to prevent bacterial growths, and the infusion allowed to stand for ten days. The solution was then evaporated to a syrupy consistency, when maltose slowly crystallised out which was purified by being dissolved in alcohol and recrystallised. It separates from an aqueous solution in needle form, and contains one equivalent of water of crystallisation; but when separated from alcoholic solution it is anhydrous. It is a white substance, soluble in water, but sparingly soluble in alcohol. It reduces Fehling's solution in a degree equal to about two-thirds of its weight of dextrose. This reduction is stated by O'Sullivan to be equal to 65 per cent. of its weight of dextrose, but Brown and Morris state the percentage to be equal to $K_{3\cdot86} = 61$, which, however, is practically the same thing, when allowance is made for the fact that O'Sullivan employed the factor 3·85 in place of the now commonly used factor 3·86.

According to Soxhlet, with an excess of undiluted Fehling's solution and an approximately 1 per cent. solution of maltose, 100 parts of maltose invariably yield 127·3 parts of cupric oxide, or 113 parts of copper. A series of tables for the estimation of maltose have been prepared by Kjeldahl.[3]

The optical activity of maltose is $[\alpha]_{D\,3\cdot86} = 135\cdot9$. It is converted into dextrose when heated for a short time with dilute mineral acid, the best temperature for the hydrolysis being 176° F. (80° C.) to 194° F. (90° C.). Malt extract or diastase is unable to effect any change in it, whilst in contact with yeast, under suitable conditions, it is hydrolysed to dextrose under the influence of a special enzyme contained within the yeast termed *maltase* or *glucase*, and is further decomposed with the production of from 51 to 52 per cent. of alcohol. When in company with dextrose the latter is fermented first.

A hot solution of maltose, heated with phenyl-hydrazine, forms osazone which separates on cooling as yellow needles, and which melt and decompose at a temperature of 390° F. (198·8° C.).

[1] *Ann. Chim. et Phys.*, xi. 379.
[2] *Jnl. Chem. Soc. Trans.*, xxv. 579.
[3] *Med. Carlsb. Lab.*, 1895, 1.

E. THE POLYSACCHARIDES.

+ *Malto-dextrins* $(C_{12}H_{22}O_{11})_n$.
$(C_{12}H_{20}O_{10})_n$.

Raffinose $C_{18}H_{32}O_{16}$.

+ *Malto-dextrins* $(C_{12}H_{22}O_{11})_n$.
$(C_{12}H_{20}O_{10})_n$.

The transformation products in a starch conversion possessing an $[\alpha]_D$ 150·3° consist of :—

Maltose . . .	80·8 per cent.
Dextrin . . .	19·2 ,,
	100·0

The optical activity of a starch transformation effected by unrestricted diastase falls rapidly from $[\alpha]_D$ 202°, representing the original soluble starch, to $[\alpha]_D$ 150·3 representing a so-called complete conversion, and when it reaches this stage the velocity of the transformation change is checked; $[\alpha]_D$ 150·3 therefore represents a well-defined point in the hydrolysis of starch. An equation representing this change, commonly called the "No. 8" equation, is given thus :—

$$\underset{\text{Starch.}}{[5(C_{12}H_{20}O_{10})]} + \underset{\text{Water.}}{4H_2O} = \underset{\text{Maltose.}}{4C_{12}H_{22}O_{11}} + \underset{\text{Stable dextrin.}}{(C_{12}H_{20}O_{10}}$$

This equation represents that four-fifths of the starch molecule is converted into maltose and one-fifth into stable dextrin; but the amount of maltose in the starch transformation products is not four-fifths the weight of these products, owing to the fixation of water during hydrolysis, hence the proportion 80·8 maltose to 19·2 dextrin in the products of a complete conversion.

The starch molecule is, however, much larger than $[5(C_{12}H_{20}O_{10})]$.

Brown and Millar[1] bring forward evidence to show that the molecule of stable dextrin is $[20(C_{12}H_{20}O_{10})]$, and therefore that the molecule of soluble starch must be at least five times as large. According to this view, the conversion of soluble starch into maltose and dextrin is represented as follows :—

$$\underset{\text{Soluble starch.}}{\left\{\begin{matrix}(C_{12}H_{20}O_{10})_{20}\\(C_{12}H_{20}O_{10})_{20}\\(C_{12}H_{20}O_{10})_{20}\\(C_{12}H_{20}O_{10})_{20}\\(C_{12}H_{20}O_{10})_{20}\end{matrix}\right.} + 80H_2O = \underset{\text{Maltose.}}{80C_{12}H_{22}O_{11}} + \underset{\text{Stable dextrin.}}{[20(C_{12}H_{20}O_{10})]}$$

[1] *Jnl. Chem. Soc.*, 1899, lxxv. 317.

The so-called "stable" dextrin, although it strongly resists the action of diastase, is eventually hydrolysed to maltose and dextrose if the action of the diastase is very prolonged; but the velocity of the action is exceedingly slow as compared with the velocity of hydrolysis of starch to maltose and dextrin.

Raffinose $C_{18}H_{32}O_{16}$.

Raffinose was discovered by O'Sullivan as a constituent, to a small extent, in barley; its common origin, however, is the sugar-beet. The formula is $C_{18}H_{32}O_{16} + 5OH_2$, and it crystallises in small needles or prisms which readily dissolve in water but are only slightly soluble in alcohol and possess but a faint sweetness.

It does not reduce Fehling's solution, its rotatory power being $[a]_{D\,3\cdot86} = 104\cdot5°$.

When heated for a short time with dilute mineral acid, it splits up into equal molecules of levulose and of a disacharide isomeric with lactose, called "melibose." This latter substance, on prolonged treatment with dilute acid, splits up into galactose and glucose in equal molecules.[1]

On fermentation, raffinose behaves differently with various yeasts; some are able to hydrolyse and ferment it completely, others only partially invert it into melibose and levulose, the latter sugar being alone fermented.

It is not known whether raffinose has any important influence upon worts or beers.

a and *β* Amylan.

These bodies were found by O'Sullivan in barley, wheat and rye. Barley contains about 2 per cent. of *a-amylan* and about 0·3 per cent. of *β-amylan*.

They were obtained from barley by extracting the cereal first with alcohol and then with water. The aqueous solution was concentrated by evaporation, and strong alcohol added, which precipitated the two bodies. The precipitates were treated with cold water, which dissolved out the *β-amylan* and left the *a-amylan*. The latter was afterwards dissolved in dilute hydrochloric acid, and precipitated therefrom by alcohol.

Both these bodies are levo-rotatory, and have the following opticities:—

a-amylan . . . $[a]_j = -24°$
β-amylan . . . $[a]_j = -73°$

Hydrolysis by dilute acids converts them into glucose.

[1] *Berichte*, xxii., 1678 and 3118.

Gum.

Lintner separated a small amount of gum from beer, and a similar, if not identical, substance was afterwards found in barley, malt, straw, bran, and "spent" grains. The gum forms very viscous solutions which are very difficult to filter. The gum is dextro-rotatory, does not reduce Fehling's solution, and is precipitated by lead acetate. Like many other gums, it gives a cherry-red colour with phloroglucin and hydrochloric acid, also a bluish-green colour with orcein and hydrochloric acid, its presence in beer being demonstrated by these reactions.

Lintner and Düll[1] have shown that the gum may be regarded as galactoxylan; for when boiled with dilute acid it is resolved into galactose and xylose. It is evidently formed by the union of a molecule of galactose with a molecule of the penta glucose sugar, *xylose*, with the elimination of a molecule of water, thus:—

$$\underset{\text{Galactose.}}{C_6H_{12}O_6} + \underset{\text{Xylose.}}{C_5H_{10}O_5} - \underset{\text{Water.}}{H_2O} = \underset{\text{Galactoxylan.}}{C_{11}H_{20}O_{10}}.$$

PROTEIDS OR ALBUMINOIDS AND ENZYMES.

Serum, Fibrin, Egg, Casein, and Plant Albumins—Molecular Constitution of the Proteids—Effects of Hydrolysis on the Proteids—Chemical Reactions of the Proteids—Members of the Proteid Groups—The Proteids of Barley—The Proteids of Malt—Enzymes or Hydrolysts—Chemical Composition of the Enzymes—Enzyme Groups—Action of Proteolytic Enzymes on the Proteids.

Diastase, Glucase or Maltase, Cytase, Invertase, Zymase.

The proteids or albuminoids, otherwise spoken of as protenaceous, nitrogenous, or albuminous bodies, form the chief part of the solid constituents of the blood, muscles, nerves, glands, and other organs of animals. They occur in small quantities in almost every part of vegetables, and in large quantities in the seeds, and in fact enter so largely into the composition of organic substances that they have been regarded as building up the animal and vegetable worlds. They are of great importance to man's existence; both plants and animals lay up reserves or stores of them in various parts of their tissues for contingent use, so that should their food supplies be suddenly withdrawn, neither the plant nor animal would immediately die, but would live for a time

[1] *Zeit. f. angew. Chem.*, 1897, 538.

on its reserves. Before these reserves, however, can be made available for the operations of nutrition, they must first be converted from their inert and mostly insoluble state into a state of solution and adaptability to circulate in the nutritive fluid which constitutes the alimentary atmosphere or environment of the protoplasmic elements. We shall hereafter more readily perceive how these operations are performed, so for the present may merely look to the characteristics of a few specially studied proteid bodies.

Albumin.

Of the various forms of albumin we have *serum albumin*, *fibrin albumin*, *egg albumin*, and *plant albumin* or *gluten*.

Serum albumin is the most abundant albuminous substance in animal bodies. It may be obtained tolerably pure from blood-serum by precipitation with lead acetate, washing with water, suspending the precipitated lead compound in water, and decomposing it with carbon-dioxide; then, by filtration, a very cloudy solution of albumin is obtained. The albumin may now be precipitated from this solution by the addition of alcohol which in time coagulates it. It is not precipitated by weak mineral acids in small quantity; but large quantities of acid precipitate it immediately, nitric acid acting most strongly. It forms a yellow, elastic, transparent substance which, when perfectly dry, can be heated to 100° F. (37·7° C.) without change. The substance to which the clotting of blood is due is termed *fibrin*. It is insoluble in water, sparingly soluble in dilute acids and alkalies and in neutral saline solutions. It may be obtained by washing blood-clots, or more readily by stirring with a bundle of twigs blood just shed, before it has had time to clot. The fibrin, which adheres in layers to the twigs, may then be stripped off and washed till perfectly white.

Egg albumin or white of egg differs from serum albumin by gradually giving a precipitate when agitated with ether, whilst oil of turpentine coagulates it. A characteristic between serum and egg albumin is that the former is easily dissolved by nitric acid whereas the latter is only dissolved with difficulty therein. The so-called vitellin contained in solution in the yolk of egg is a mixture of albumin and casein. If a drop of egg albumin is allowed to fall into a saturated solution of resorcinol, the drop of albumin, at first transparent, becomes gradually opaque, and finally white like a hailstone. It gradually falls through the liquid, lengthens itself out to a band, becomes broader and

broader, and finally reaches the bottom. It has the appearance of a bacteriological culture. If the liquid is now shaken it falls to the finest powder, and is so disseminated in the froth that it appears to have dissolved. The same effects are produced however dilute the egg albumin may be.

Casein or legumin, sometimes termed alkali-albumin, or albuminate, according to whether the substance is derived from animal or vegetable sources.

An example of casein is the flocculent substance which separates when milk becomes acid, whilst legumin occurs in peas, beans, etc.

Casein occurs most plentifully in the milk of animal feeders, and is best obtained from milk by precipitating with crystalline magnesium sulphate, filtering and washing with concentrated solution of sodium chloride, and dissolving the precipitate in water; the butter is then filtered off, and the clear solution precipitated by dilute acetic acid. Dried casein and albuminate are yellow, transparent, and hygroscopic; they swell in water but do not dissolve. They dissolve easily in alkaline water when placed in it in a flocculent state. The precipitate which forms, on neutralising the alkaline solution, dissolves easily in an excess of acetic or hydrochloric acid.

By fusion with potassium hydrate, casein yields valeric and butyric acids, besides other products.

Plant albumin or gluten is a substance analogous to the fibrin previously described; it occurs as an insoluble substance in plants, especially in the seeds of cereals and grasses.

When wheat flour is lixiviated with water, a tough, coherent, elastic mass is left behind, which can be pulled out into strings. This is the gluten, or the body which gives the coherent character to the dough, and the presence of which confers the property of enabling it to yield a light, porous bread. Gluten cannot be obtained in this way from the meal of any other grain. It is insoluble in water, becomes dark by exposure to the air; whilst dried at a low temperature, it assumes a yellowish-brown colour and becomes horny, and when treated with strong alcohol it assumes an earth-like appearance. It may be dissolved by hydrochloric acid and dilute alkalies, and is precipitated from these solutions by mineral salts and acetic acid.

Molecular Constitution of the Proteids.

All proteids contain the elements carbon, hydrogen, nitrogen, and oxygen, whilst occasionally they contain sulphur and phos-

phorus. Our knowledge of their composition is, however, very imperfect, and at present it is assumed that their molecule is extremely complex.

Stohmann and Langbein assert that the formula of crystallised proteid is $C_{720}H_{113}N_{218}S_5O_{248}$, equivalent to a molecular weight of 16,954, whilst Sabanejeff obtained a molecular weight of 15,000 by Raoult's cryoscopic method. This method, however, is admitted to be inapplicable to the accurate determination of the molecular weights of colloid bodies, hence the figures cannot be accepted as of definite value, but merely as a confirmation of the high molecular weight of these bodies. Many investigators have obtained compounds of proteids with inorganic salts; thus a copper salt obtained approximates to the formula

$$C_{264}H_{322}N_{52}O_{66}S_2,$$

whilst with magnesium and other salts the formula

$$C_{292}H_{481}N_{90}O_{83}S_2$$

has been indicated.

Effects of Hydrolysis on the Proteids.

By the action of certain hydrolysing agents such as superheated steam, dilute mineral acids, caustic alkalies or enzymes, the latter of which are dealt with hereafter, the large proteid molecule is split up into much smaller and less complex molecules. From a study of these we have obtained some general knowledge as to the nature of the molecular groups which enter into the composition of the proteids. When the proteids are acted upon by enzymes, the splitting-up process does not extend nearly so far, the molecules of the bodies produced being much nearer in size to that of the original proteid molecule.

Of such a nature are the proteoses and peptones.

The nature of the hydrolysis may be conveniently stated as follows:—

Proteid—Albumin.
|
Proteoses.
|
Peptones.
|
Amides.

All the amides are of a crystalline nature and eminently diffusible in plants; and it is extremely probable that the amides in the living plant form a portion of the materials from which the

proteids are constructed, the amides themselves being probably produced by a combination of the deoxidation products of the chlorophyll cells with nitrogen, derived from the nitrates and ammonia contained in the fluid contents of the cells. Whether this conjecture is right or wrong, we know that numerous amide bodies and also various products resulting from their hydrolysis exist in plants. We are also aware that by the action of acids, alkalies, or steam, proteids or albumins may be decomposed to proteoses and amides, and that the latter bodies are further hydrolysed. Thus by the action of boiling hydrochloric acid to which a little stannous chloride is added, amides produce, more or less, the following substances:—

Leucine.
Tyrosine.
Aspartic acid.
Glutamic acid.
Glutaminic acid.
Lysine.
Arginine.
Histidine.
Lysatine.

When barium hydroxide is used as the hydrolysing agent, in addition to the formation of the above-mentioned products, much ammonia and carbon-dioxide are evolved; and these gases are given off in the proportion of one molecule of water and one of urea, $CO\begin{matrix}NH_2\\NH_2\end{matrix} + OH_2 = CO_2 + 2NH_3$.

Taking the principal amide substances, we have the following:—

Leucine or *amido-caproic acid.*

$$C_6H_{13}NO_2 = \begin{matrix}CH_3\\CH_3\end{matrix}\!>\!CH{\cdot}CH_2{\cdot}CH\!<\!\begin{matrix}NH_2\\COOH.\end{matrix}$$

This amide is usually the normal product in every energetic decomposition of proteid matter; it is obtainable in considerable amount by the hydrolysis of horn by acids, and by the action of trypsin upon most proteids. It is found in germinating seeds and is present in germinated barley and in malt worts. In a state of purity it consists of brilliant, silvery-white plates, which melt at 338° F. (170° C.). It is soluble in water, and its aqueous solutions are dextro-rotatory. It is only slightly soluble in alcohol. It has been prepared synthetically. When

treated with nitrous acid it yields up the whole of its nitrogen thus:—

$$\underset{\text{Leucine.}}{C_6H_3NO_2} + \underset{\text{Nitrous acid.}}{HNO_2} = \underset{\text{Hydroxy-caproic acid.}}{C_6H_{12}O_3} + \underset{\text{Water.}}{OH_2} + \underset{\text{Nitrogen.}}{2N}$$

Tyrosine or *parahydroxy-phenylamido-propionic acid*

$$C_9H_{11}NO_3 = C_6H_4\left\langle\begin{matrix} OH \\ CH_2 \end{matrix}\right. \quad CH\left\langle\begin{matrix} NH_2 \\ COOH \end{matrix}\right.$$

is also produced in the decomposition of all proteids, with the exception of gelatine, and has frequently been detected in germinated seeds. When pure, it crystallises in brilliant silky needles which are not readily soluble in water, and insoluble in alcohol. An aqueous solution is lævo-rotatory, but a dextro-rotatory modification is known.

It is found in the liver and other organs, and among the excretory products of yeast, and frequently accompanies the decomposition of proteids by the putrefactive action of bacteria.

Treated with nitrous acid, it yields up nearly the whole of its nitrogen.

Asparagine or *amido-succinamide.*

$$C_4H_8N_2O_3 = COOH\left\langle\begin{matrix} CH \\ NH_2 \end{matrix}\right\rangle CH_2{\cdot}CONH_2.$$

So called because it was first found in asparagus sprouts. This substance, although probably not resulting from the breaking down of proteids by proteohydrolysts, is of great importance, since it is found in considerable amount in many plants and germinating seeds. It has not yet been isolated from malt, but occurs in considerable quantity in the rootlets. It is supposed that the formation of this compound in living plants is, in reality, a result of synthesis rather than decomposition; that is to say, as previously mentioned, it makes an early stage in the building up of the complex albuminoids rather than a final stage in the retrograde decomposition by proteolysis.

Although the existence of asparagine in malt and wort is uncertain, there is little doubt that its presence would be of considerable benefit. The extraordinary stimulating effect of this substance upon the activity of diastase has been investigated by Effront and Fernback, and its high value as yeast nutriment has been fully demonstrated by Stern.

In a state of purity it forms large colourless crystals, soluble in

water, but insoluble in alcohol. Its aqueous solutions are lævo-rotatory, its specific rotatory power being $[\alpha]_D = -6 \cdot 23°$. A dextro-rotatory asparagine which rotates the same angle in the opposite direction has, however, been found in tares. The dextro-rotatory body has a distinctly sweet taste; the lævo-rotatory is almost tasteless. When heated with dilute acids, asparagine is readily transformed into aspartic acid and ammonia, thus:—

$$\begin{array}{ccccccc} C_4H_8N_2O_8 & + & OH_2 & = & C_4H_7NO_4 & + & NH_3. \\ \text{Asparagine.} & & \text{Water.} & & \text{Aspartic acid.} & & \text{Ammonia.} \end{array}$$

When acted on by nitrous acid, it yields half of its nitrogen in the gaseous state as follows:—

$$\begin{array}{ccccccccc} C_4H_8N_2O_8 & + & NHO_2 & = & C_4H_6O_5 & + & NH_3 & + & 2N. \\ \text{Asparagine.} & & \text{Nitrous acid.} & & \text{Malic acid.} & & \text{Ammonia.} & & \text{Nitrogen.} \end{array}$$

A method for the estimation of asparagine and the amides generally has been based on this reaction by Sachasse.[1] Asparagine is precipitated by mercuric nitrate, and the compound so obtained may be decomposed into asparagine and mercuric sulphide by the action of hydrogen sulphide.

Aspartic acid or *amido-succinic acid.*

$$C_4H_7NO_4 = \begin{array}{l} CH\left\langle\begin{array}{l}NH_2\\ COOH\end{array}\right. \\ CH_2\text{—}COOH. \end{array}$$

This is the crystalline body previously referred to; it is slightly soluble in water, rotates the polarised ray to the left or right, according as the asparagine from which it is obtained is lævo- or dextro-rotatory. Treated with nitrous acid, it yields up the whole of its nitrogen thus:—

$$\begin{array}{ccccccccc} C_4H_7NO_4 & + & HNO_2 & = & C_4H_6O_5 & + & OH_2 & + & 2N. \\ \text{Aspartic acid.} & & \text{Nitrous acid.} & & \text{Malic acid.} & & \text{Water.} & & \text{Nitrogen.} \end{array}$$

Among other amide compounds resulting from the decomposition of the proteids, the following may be enumerated, which have a more or less important bearing upon the subject:—

Glutamic acid or *amido-glutaric acid.*

$$C_5H_9NO_4 = CH_2\left\langle\begin{array}{l} CH\left\langle\begin{array}{l}NH_2\\ COOH\end{array}\right. \\ CH_2\text{—}COOH. \end{array}\right.$$

[1] *Agricult. Chem.*, 390.

Glutaminic acid.

$$C_5H_{10}N_2O_3 = CH_2 \left\langle \begin{array}{l} CH_2{\cdot}CONH_2 \\ CH \left\langle \begin{array}{l} NH_2 \\ COOH. \end{array} \right. \end{array} \right.$$

And the hexone bases :—

Lysine or *diamido-caproic acid.*

$$C_6H_{14}N_2O_2 = CH_2NH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH \left\langle \begin{array}{l} NH_2 \\ COOH \end{array} \right.$$

a dextro-rotatory substance from hydrolysis of casein.

Arginine or *diamido-valeric acid.*

$$C_6H_{14}N_4O_2 = NH = C \left\langle \begin{array}{l} NH_2 \\ NH \end{array} \right\rangle CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH \left\langle \begin{array}{l} NH_2 \\ COOH. \end{array} \right.$$

Histidine.—$C_6H_9N_3O_2$.

Lysatine.—$C_6H_{13}N_3O_2$.

In addition to the above compounds, glycocoll (*amido-acetic acid*) $CH_2 \left\langle \begin{array}{l} NH \\ COOH \end{array} \right.$ sometimes results from proteolytic decomposition, and has been detected in many plants.

Besides the important amide products, resulting from the decomposition of the proteids by hydrolysis, either by acids or enzymes, there are numerous bodies which result from the breaking down of albuminoid substances by the aid of living organisms ; these are frequently excretory products such as :—

Xanthine . . .	$C_5H_4N_4O_2$.
Sarcine . . .	$C_5H_4N_4O$.
Guanine . . .	$C_5H_5N_5O$.

The above and other xanthine bases are found among the excretory products of yeast and micro-organisms of other description. In addition to these or similar substances, besides the normal products of proteohydrolysis, many pathogenic organisms form either products of a phylacteric nature, such as protective serums, or of a poisonous nature, such as the *toxins*, which, together with the *ptomaines* formed by certain kinds of putrefactive bacteria, partake of the nature of alkaloids.

Chemical Reactions of the Proteids.

The proteids give the following characteristic reactions, which may readily be observed with a solution of egg albumin, made by

mixing a small quantity of white of egg, which contains about 12 per cent. of albumin, with water, and filtering.

Precipitation by Nitric Acid.—When strong nitric acid is added to an aqueous solution of any of the proteids, a white precipitate forms, which turns yellow on heating the liquid. The addition of ammonia to the mixture, after it has become cold, causes the yellow precipitate to become orange-coloured.

Biuret Reaction.—On adding a few drops of a dilute solution of cupric sulphate to an aqueous solution of a proteid, and afterwards a few drops of a strong solution of caustic soda, a colour is developed which varies with the different classes of proteid. The proteids give a violet colour; the proteoses, a reddish-violet; the peptones, a rose-red.

Millon's reagent[1] yields a white precipitate which becomes reddish on boiling the fluid.

Evolution of Ammonia.—The proteids when strongly heated evolve ammonia, produced under the destructive action of heat. The ammonia may be recognised by its changing the colour of a piece of moist red litmus paper to blue. A smell resembling that of burnt hair is also given off during the heating.

Formation of Cyanides.—The proteoses, when heated with metallic sodium, yield sodium cyanide. The presence of this compound can be detected by extracting the mass with water, adding a few drops of a solution of ferrous sulphate containing a little ferric sulphate, and digesting for a short time. On the addition of hydrochloric acid a blue or bluish-green colour is produced if proteids are present.

Members of the Proteid Groups.

By means of the solvent power of various saline solutions it has been found possible to isolate the proteids from the substances with which they are associated, and to effect a fairly complete separation of the various members of the proteid groups from one another by reason of the further property possessed by certain salts of throwing out of solution certain members of the groups when the solution is saturated with the particular salt.

This latter process is known as "salting out," and by the

[1] **Millon's Reagent.**—Prepared by gently warming mercury with an equal quantity of strong nitric acid till it dissolves, then diluting the liquid with twice its bulk of water, and leaving the precipitate to settle. The clear supernatant liquid is the reagent.

employment of this method the various plant proteids have been divided into the following groups:—

Albumins.—Soluble in water, coagulated by heat.

Gliadin and Hordein.—Slightly soluble in water, readily soluble in 70 per cent. alcohol.

Glutenin.—Slightly soluble in hot water and hot alcohol, soluble in 0·1 per cent. solution of caustic potash and in 0·2 per cent. hydrochloric acid; insoluble in saline solutions.

Gloubulins-Vitellins.—Insoluble in water, soluble in dilute saline solutions, coagulated in great part by heat.

Gloubulins-Myosins.—Insoluble in water, soluble in dilute saline solutions, precipitated by sodium chloride, coagulated by heat.

All these bodies, excepting the latter (gloubulins), are obtained in an amorphous condition, in which state they probably exist in plants. Many of the gloubulins have been obtained in a crystalloid form, and to some extent exist in this condition in seeds.

The Proteids of Barley.

Ritthausen[1] succeeded by fractionation with alcohol in differentiating three proteid bodies from wheat-flour, viz., gluten-fibrin, gliadin, and mucedin, and a fourth, gluten-casein, which was insoluble in alcohol but soluble in dilute alkali. He considered that these four bodies constituted the gluten of wheat, and that it was the gliadin which formed the binding material. This body was found to be absent in the flour of those grains which left no gluten behind on washing.

Ritthausen also considers that these are the bodies belonging to the gluten group in barley, viz., gluten-fibrin, gluten-casein, and mucedin.

Osborne[2] finds that there are only two: hordein, which is apparently identical with Ritthausen's mucedin, but which has almost the same physical and chemical properties as the gliadin obtained from wheat, though it differs from it in composition; the second being the insoluble proteid which it was found impossible to isolate.

Mulder[3] found a barley to contain 6 per cent. of albumin and plant gluten; he obtained the latter by extracting the ground barley with hot alcohol.

Von Bibra[4] considers that the proteids of barley are albumin,

[1] *Die Eiweisskörper.*

[2] *Jnl. Amer. Chem. Soc.*, xv. 392.

[3] *Ann. Chim. et Phys.*, 306.

[4] *Die Getreidearten*, 204.

plant gluten, and casein, but gives no particulars concerning these substances.

Kreusler found that an aqueous extract of ground barley contained an albumin which coagulated on boiling, and that hot 75 per cent. alcohol dissolved a substance which could be subsequently separated into three proteids—gluten-casein, gluten-fibrin, and mucedin, which were supposed to be identical with the bodies having the same names, which Ritthausen had isolated from wheat.

According to Osborne,[1] to whom we are indebted for an elaborate and extensive series of investigations on the proteids of various grains and seeds, barley contains the following proteid bodies:—

	Per cent.
Leucosin (albumin) . .	0·30
Proteose / Edestin (globulin) } . .	1·95
Hordein	4·00
Insoluble proteid . . .	4·50
Total . . .	10·75

whilst the average percentage composition of a large number of analyses gave the following:—

	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.
Leucosin . . .	52·81	6·78	16·62	1·47	22·32
Edestin . . .	50·88	6·65	18·10	24·37	
Hordein . . .	54·29	6·80	17·21	0·83	20·87
Insoluble proteid .	Unknown.				

The Proteids of Malt.

During the process of malting, the proteids of barley undergo considerable modification, a large portion which are insoluble in water becoming soluble as the barley germinates. This arises chiefly from the breaking down of the proteids into proteoses.

The proteids of malt have been investigated by Osborne and

[1] *Report of the Connecticut Agricultural Experimental Station*, 1892.

Campbell,[1] who, by employing similar processes to those which they adopted in their investigations on the proteids of barley, obtained the following bodies :—

Bynedestin.—A globulin, soluble in dilute solutions, and therefore passing into the aqueous extract of malt. It appears to replace the original edestin of the barley, from which it differs in composition, since bynedestin contains about 2 per cent. more carbon and 3 per cent. less nitrogen. Its percentage composition is—

Carbon	53·19
Hydrogen	6·69
Nitrogen	15·68
Sulphur	1·25
Oxygen	23·19

Leucosin.—Identical in composition and properties with the albumin of the same name contained in barley.

Protoproteose 1.—Has the same composition as leucosin, from which it is impossible to effect a complete separation. The proteose is precipitated from its aqueous solution by adding an equal weight of alcohol.

Protoproteose 2.—Less readily precipitable than No. 1, by alcohol, its percentage composition being—

Carbon	50·63
Hydrogen	6·67
Nitrogen	16·69
Oxygen and sulphur . .	20·01

Deuteroproteose.—A body inseparable from non-proteid impurities.

Heteroproteose.—A substance found in extremely small amount.

Bynin.—A body insoluble in water or saline solutions, but readily soluble in dilute alcohol, its percentage composition being—

Carbon	55·03
Hydrogen	6·67
Nitrogen	16·26
Sulphur	0·84
Oxygen . . .	21·20

Insoluble Proteid.—This, which amounts to about 3·80 per cent. of the total proteid matters, is insoluble in water, saline

[1] *Report of the Connecticut Agricultural Experimental Station*, 1896, 239.

solutions, or alcohol; and consequently it is impossible to study its composition or determine its properties. A sample of malt which contained altogether 7·84 per cent. of proteid matters, yielded the following quantities of these substances, as far as they could be separated:—

	Per cent.
Proteids, insoluble in salt solution or alcohol .	3·80
Bynin, soluble in dilute alcohol	1·25
Bynedestin, leucosin, and proteoses (coagulable) .	1·50
Bynedestin, soluble in water and salt solution (uncoagulable)	1·29
Total proteids . . .	7·84

These results show that during germination the proteids of barley undergo extensive changes before acquiring the properties of proteoses; that hordein disappears, and an alcohol-soluble body of entirely different composition takes its place; that edestin also disappears, and a new globulin is formed very different both in composition and properties.

The albumin, on the other hand, appears to be unchanged in character, but its quantity is increased. It is to be noted that hordein and edestin are both replaced by proteids much richer in carbon and poorer in nitrogen.

Enzymes or Hydrolysts.

Enzymes or hydrolysts exist in all living organisms whether of animal or vegetable origin, and are remarkable nitrogenous bodies either actually albuminoids or very closely allied to them.

There are numerous varieties, each having its special correlative alimentary principle or group of principles, on which, under certain conditions (an absolutely necessary one being the presence of water), it is capable of acting. Diastase, for instance, acts on amylaceous substances and cane-sugar; whilst pepsin and trypsin act only on the azotised principles. The emulsive ferment of the pancreas is only capable of acting on fatty bodies, and the inversive ferment of yeast and of the small intestine has no activity except on cane-sugar.

These nitrogenous bodies are often spoken of as the "unorganised ferments" or "enzymes,"[1] and the transformations they effect

[1] The word "enzyms" was first proposed by Kühne. Roberts afterwards adopted the word into English with a slight change of orthography, terming it "enzymes."

"fermentative processes." In 1890, however, Armstrong suggested more scientifically correct terms, viz., as in the changes brought about by these bodies water is almost invariably assimilated or added to the molecule of one or both of the newly formed substances, the process is one of, and should be designated, "hydrolysis," and the agents concerned in the action "hydrolysts." Thus by combining with the word "hydrolyst," the name of the substance on which each particular enzyme acts, we obtain a distinctive name for each class. Thus the enzymes which act upon starch are now called amylo-hydrolysts; those that act on proteids, proteo-hydrolysts, etc.

Chemical Composition of the Enzymes.

It is doubtful whether any of the preparations of the enzymes obtained by different investigators have been of a sufficient degree of purity to permit of accurate determination of their chemical composition.

Lintner, working with the diastase of malt, obtained a solution possessing a high diastatic power having the following composition:—

Carbon	46·66
Hydrogen	7·35
Nitrogen	10·41
Oxygen	34·46
Sulphur	1·12

A substance having this composition differs from albumin, to which the enzyme bodies are generally closely allied, albumin on analysis giving the following percentage composition:—

Carbon	53·02
Hydrogen	6·84
Nitrogen	16·80
Oxygen	22·06
Sulphur	1·28

At first sight one would be disposed to consider diastase as a substance differing in a marked degree from albumin. Osborne, however, who has made a very close study of the albuminoid constituents of cereals, isolated a diastase having a much higher diastatic power than Lintner's preparation, its diastatic power being equivalent to 600° upon Lintner's scale.

The composition of this body, which is undoubtedly one of

the purest preparations of the enzymes which has been obtained, is as follows:—

Carbon	52·50
Hydrogen	6·72
Nitrogen	16·10
Oxygen	22·78
Sulphur	1·90

a body of this percentage composition closely agreeing with the analysis of albumin already given.

Osborne further found that this diastatic preparation had a very close resemblance to *leucosin*, an albumin which he had previously isolated from barley, wheat, rye, and malt.

Other preparations of diastase and enzyme substances have been made by different investigators, the chief of which are included in the following table by Effront, "Les enzymes":—

	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Ash.
Malt diastase . .	45·68	6·90	4·57	...	6 08
,, ,, . .	47·57	6·49	5·14	...	3·16
Invertase . .	43·10	7·80	4·30	...	6·10
,, ...	43·90	8·40	6 00	0·63	...
,, ...	40·50	6·90	9 30	...	...
Ptyalin . . .	43·10	7·80	11·86	...	6·10
Trypsine . . .	52·75	7·50	16·55	...	17·70
Pepsine . . .	53·20	6·70	17·80	...	...
Pancreatin . .	43·60	6·50	13·81	0·88	7·04
Emulsin . . .	43·06	7·20	11·52	1·25	...
,, ...	48·80	7·10	14·20	1·30	...

It will be seen from the foregoing table that considerable differences exist between the various enzyme bodies, these differences being due to imperfectly purified substances. The difficulty of obtaining these bodies free from ash and carbohydrate matters, with which they are always contaminated, being the cause.

All the enzymes when in solution are extremely susceptible to elevated temperatures, their power being weakened, and if the heating be continued they are wholly destroyed, the destruction being usually accompanied by separation of the albuminoid in an insoluble form, since many of them appear to belong to the group of coagulable proteids.

ENZYME GROUPS.

The enzymes may be divided into the following seven groups, in which they are arranged according to their respective specific actions :—

Group I.—**Diastatic Enzymes** :—

Diastase of secretion (malt) Translocation diastase (barley) Ptyalin (saliva)	Convert starch into maltose and dextrin.
Glucase (maize)	Converts starch finally into glucose.

Group II.—**Cyto-hydrolytic Enzymes** :—

Cytase (malt) Enzymes of seeds in which the reserve material is cellulose	Transform cellulose into sugars, such as mannose, xylose.

Group III.—**Pectin Enzymes** :—

Enzymes which convert pectinous substances into vegetable jelly.

Group IV.—**Inverting Enzymes** :—

Invertase (yeast) Invertase (malt)	Convert cane-sugar into invert sugar.
Maltase or glucase (yeast) Enzymes of the small intestine	Transform maltose into glucose.

Enzymes of yeast which degrade the intermediate dextrins into maltose ; these are especially present in wild yeasts.

Enzyme of Kephir	Inverts milk-sugar.
Probable enzyme of germinating barley	Converts maltose into cane-sugar.

Group V.—**Proteolytic Enzymes** :—

Enzymes of malt and other vegetables (sometimes called peptase), but which have not yet been *definitely* isolated	Convert proteids into proteoses, peptones, and amides.
Trypsin (pancreas)	Amido acids and hexone bases.

Pepsin (stomach) . . .	Converts proteids into proteoses and peptones, but not into amides.

Peptonising ferments secreted by many bacteria.

GROUP VI.—**Glucosidal Enzymes** :—

Emulsin (bitter almond) . .	Splits up amygdalin into oil of bitter almonds, hydrocyanic acid, and water.

Many other enzymes which have the power of hydrolysing glucosides.

GROUP VII.—**Zymase** (yeast) :—

Splits up sugar into alcohol and carbon-dioxide.

ACTION OF THE PROTEOLYTIC ENZYMES ON THE PROTEIDS.

From the preceding list showing the various groups of enzymes, we have, amongst others, those of *animal origin*, which act upon insoluble proteids as follows :—

1. The pepsin group, which act upon albumins, degrading them to proteoses and peptones.

2. The trypsin group, which act upon albumins, resolving them beyond the peptone stage, with the formation of simpler compounds, viz., amido acids and hexone bases.

There is no doubt that proteolytic enzymes exist in plants, since, as already mentioned, before the reserve materials stored up by plants can be made available for the operations of nutrition, they must first be converted from their inert and mostly insoluble state into a state of solution and adaptability to circulate in the nutritive fluid of the plants.

Group-Besanez, in 1874,[1] was the first to conclude that a proteolytic enzyme existed in germinating barley, and he considered he had extracted it not only from barley, but also from tares, hempseed, and linseed.

It is extremely doubtful, however, whether or not he detected such enzyme, and in any case his method for detecting the products of its action is open to criticism. A powerful enzyme of this nature has, however, been lately isolated in a fairly pure state from the latex of the tropical plant *Carica papaya*, so that proof is no longer wanted of its existence in plants, although it has not yet been isolated from barley or malt. Shortly after

[1] *Berichte*, 1874, 7, 1478.

Group-Besanez's investigations, Neumeister,[1] working with extracts prepared from germinating barley and green malt, obtained products from blood fibrin which he considered exhibited characteristic albumose and peptone reactions. Here the matter rested until 1899, in which year Lazynscki[2] disputed the existence of a proteolytic enzyme. In 1900, however, Fernback and Hübert[3] concluded that they had discovered it, and then followed other investigations by Windisch and Schellhorn,[4] Petit and Labourasse,[5] Weiss,[6] and finally Schidrowitz,[7] all of which endeavoured to prove the existence of a proteolytic enzyme in barley and malt.

The tests of these various investigators, however, have no real value, and up to the time of writing no proteolytic enzyme has been satisfactorily isolated from barley or malt, and on this account there is and will be, from time to time, considerable controversy as to whether barley or malt contains such an enzyme and whether any peptonising action really takes place during mashing.

When germination commences in the grain of barley, it is necessary that the starch stored up in its endosperm should be able to travel to the growing germ. This is first effected by an enzyme or amylo-hydrolyst (the translocation diastase) already present in the seed, afterwards by another hydrolyst (ordinary diatase) which is formed in considerable quantity during germination.[8] In like manner the insoluble proteids in the grain are, to a great extent, rendered soluble and diffusible, but in what manner has not yet been settled, although from what has been stated there is strong evidence of the existence of proteid-hydrolysts both in barley and malt in spite of the fact of their non-extractability therefrom.

As, therefore, we are unable to obtain a single proteolytic enzyme from barley or malt, we have to rest content with a knowledge of the action of the animal proteolytic enzymes, pepsin and trypsin, which can be obtained in an almost pure state from the stomach and pancreas of animals, and surmise, from their action, what action takes place during the germination of barley and the peptonisation of the proteids of malt during mashing.

Wort must contain proteid bodies of a nature readily assimilable

[1] *Zeit. f. Biologie*, 30 (94), 447. [2] *Zeit. f. d. gesammt. Brau.*, 22, 71.
[3] *Compt. Rend.* (4). [4] *Wochensch. Brauw.* (1900), 17, 334–452.
[5] *Compt. Rend.*, July 5 and 6, 1900.
[6] *Zeit. f. phys. Chem.*, 31, 78–97, and *Zeit. ges. Brauw.*, 1903.
[7] *Jnl. Fed. Inst. Brewing*, 1903, 361.
[8] "Researches on the Germination of some of the Gramineæ," *Jnl. Chem. Soc. Trans.*, 1890, 458–528.

by yeast, or the yeast would speedily become weak for want of food and as a consequence become incapable of properly fulfilling its functions. The albumin and globulins which occur in barley seem completely indiffusible, but the proteoses and amides are very highly diffusible.

These facts therefore show that the diffusible and assimilable bodies requisite for yeast nourishment are yielded by the proteolysis of the proteids originally contained in the barley, effected for the most part during its germination, and afterwards, to a slight extent, during the mashing process. Pepsin acts best in slightly acid media, preferably 2 per cent. hydrochloric acid, and the most favourable temperature for its action is 100° F. (37·7° C.), the "blood-heat" of most mammals. When coagulated egg albumin or other insoluble proteid is added to an acid solution of pepsin, and maintained at a temperature of 100° F. (37·7° C.) for some hours, the process of digestion is imitated, solution of the proteid taking place accompanied by a degradation of the albumin molecule to proteose and then peptone, the proportion of the latter body being dependent upon the time of digestion.

Pepsin is incapable of degrading proteids beyond the peptone stage, so that peptone must be considered the final product of its action, although there is evidence that other bodies besides proteoses and peptones are formed. These secondary products result from the action of pepsin upon a portion of the peptone, and although closely allied to this group, do not give the characteristic reactions of these bodies.

According to Dr Sykes,[1] "The proteid molecule consists of two distinct groups or radicles, from which the various products of proteolytic action are derived. The following schematic arrangement will, without going into too minute distinctions, give a general idea of the series of changes :—

Proteid
|
Syntonin —— Anti-albumin
|
Hemi-group.	Anti-group
Proteose	Hetroproteose
Deuteroproteose	Deuteroproteose
Peptone	Peptone.

[1] *Principles and Practice of Brewing*, 167–168.

"The great distinction between these two groups, which are called the hemi- and anti-groups, and which appear to exist in about equal quantities, is that the peptone of the former can be further broken down by the action of trypsin into a number of amide bodies, whilst the peptone of the latter cannot be so broken down. In every digestion a variable quantity of a substance is left undissolved, which is called anti-albumin; it is extremely resistant to the further action of the proteolytic enzyme, but, by treatment with strong solution of pepsin and acid, may be partially converted into anti-deutero-albumose, and finally into anti-peptone."

Sykes proposes [1] the following process for the estimation of the proteids in malt:—"The malt is extracted with cold water, filtered, and the albumin in the filtrate coagulated at a temperature of 140° F. (60° C.). The coagulum is filtered off and weighed. The filtrate is then boiled for a short time, when a second precipitate forms, which is filtered off and weighed. This is considered to represent the globulin, but from the recent experiments of Osborne, it is extremely doubtful if all the globulin can be separated by boiling.

"The filtrate is now evaporated to small bulk and saturated with ammonium sulphate, when a precipitate consisting of the proteoses (and probably some globulin) comes down. This is filtered off, washed with saturated solution of ammonium sulphate, dried and weighed, the weight of the ammonium sulphate, adhering to the precipitate and filter paper, being determined and deducted. The filtrate from this precipitate is then diluted with an equal quantity of water, and solution of tannic acid added. This precipitates peptone, if it be present; but no precipitate of any moment has ever been observed, which leads to the conclusion that real peptone does not occur in malt."

Szymanski considered that he had separated peptone from malt, but from the process he adopted for its isolation, the substance which he obtained and mistook for real peptone was evidently deuteroproteose.

Sykes, experimenting on the proteids of malt, was never able to detect a hetroproteose on dialysing the precipitate thrown down by ammonium sulphate.

A precipitate was always yielded on saturating malt extract with sodium chloride and adding a little acetic acid, leaving the body in solution which Szymanski mistook for peptone. In all probability, therefore, the two proteoses are proto- and deutero-proteose.

[1] *Jnl. Fed. Inst. Brewing*, vol. iv. 173.

Diastase.

The diastatic enzymes are of the greatest importance and interest in brewing and malting, from the fact that they embrace the numerous bodies which have the power of liquefying and saccharifying starch.

Of this group, *malt diastase* is the best known, for the reason that it has received the most attention from scientific investigators.

In 1811, Kirchoff found that the albumin contained in barley is capable of acting upon soluble starch and producing a crystallisable sugar similar to that produced when starch is boiled with dilute acid; and he noticed that this action is greatly intensified if the barley is germinated or malted.

Payen and Persoz, in 1833, were the first, however, to isolate the soluble "ferment" and to identify it as the principle which is capable of exercising such a powerful action upon starch. They gave it the name diastase, which it still retains. This name was given on account of the agent's supposed property of separating the interior of the starch granule (granulose) from its outer membrane (amylo-cellulose), but, as is now well known, this is not strictly true, and furthermore erroneous ideas prevailed as to the nature of the sugar produced by its action.

The isolation of diastase from malt was undoubtedly a most important discovery, but little further advance was made until O'Sullivan, in the course of his researches upon the products of the hydrolysis of starch, again isolated it in a fairly pure state, and from his study of its specific action under varying conditions of temperature, evolved many of the principles which are now well known to govern its action.

Diastase has been detected in almost all animal tissues and vegetable substances; thus Payen and Persoz detected its presence in many *germinating* seeds, such as wheat, maize, rice, oat, potato tubers, and in the buds of *Ailanthus glandulosa*; Kossmann and Kranch found it in foliage and vegetable shoots; whilst we owe to Kjeldahl the observation, confirmed by Brown and Morris, of the presence of diastase, albeit in sparing quantity, among *ungerminated* seeds.

Mulder mentions its occurrence in saps which contain starch, Hansen found it in the sap of *Ficus carica*, and Gonnermann detected it in *Beta vulgaris*. To this we have to add that Duclaux has shown the common moulds, *Aspergillus niger* and *Penicillium glaucum*, produce it in abundance; that Bourquelot has conducted a complete series of researches, having as its object the

discovery of diastatic ferments in mould fungi generally, and we perceive how universal is the occurrence and how widely distributed are amylo-lytic ferments in the vegetable world. Yeast itself is said to contain a small quantity of diastase, whilst certain *Mucors*, also thus endowed, are able to directly ferment starch. The well-known Tonkin yeast, and even the better known Koji yeast, the micro-organism employed in the production of the Japanese beverage, *Saki*, afford peculiar symbioses of diastase-producing mould fungi with alcohol-producing saccharomycetes; in the first-named case a mould, *Amylomyces Rouxii*, being associated with a pastorianus yeast, and, in the second instance, *Aspergillus oryzæ* producing the taka diastase, whilst the alcohol is formed by the action of some true alcoholic ferment. Diastase has also been isolated from bacteria, investigators such as Wood, Wortmann, Perdrix and others having succeeded in proving its existence in organisms such as the *Cholera vibrio*, *B. mesentericus vulgaris*, *B. termo*, and in fact practically every micro-organism is now believed to secrete an enzyme capable of dissolving starch.

Plants possess three distinct diastases, called respectively secretion diastase, translocation diastase, and cytase; these are respectively dealt with hereafter under Cytase, where diastatic action from the maltster's point of view is detailed. From the brewer's point of view, however, malt diastase is the agent which, under suitable conditions during mashing, saccharifies or transforms soluble starch into dextrin, maltose, and intermediate malto-dextrins. There is a further part played by diastase which it is also necessary to remember, viz., the strobiles of the hop plant contain active diastase which exercises an important function in "hopping down," the conditioning of cask beer being brought about to some extent by its hydrolysing action on the residual malto-dextrins.

O'Sullivan obtained active preparations of diastase by digesting finely ground malt, preferably low dried, with cold water for several hours. The mass is then placed in a press and the resulting extract filtered. On adding 80 per cent. alcohol to the clear filtrate, a precipitate containing diastase is formed, which is collected, washed with alcohol and finally with ether, and then dried as quickly as possible in a *vacuo* desiccator over sulphuric acid. During manipulation there is a liability, if the manipulation be unduly prolonged, for the diastase to become converted into a hard mass which is only partially and with difficulty soluble in water.

Lintner has devised a better method for preparing diastase from malt, as follows:—

One part of air-dried malt is digested for 24 hours with two to four parts of 20 per cent. alcohol. The liquid is filtered and precipitated by two or three times its own volume of absolute alcohol. The precipitate, rapidly collected on a filter, is crushed in a mortar with absolute alcohol, filtered, again washed with absolute alcohol, then with ether, and dried *in vacuo*. When the operation is performed properly, it yields a white powder which is readily soluble in water, giving an almost clear solution.

This method of precipitation by alcohol was first employed by Payen, to whom we owe the earliest important work on diastase. He had noticed what is also observed with Lintner's diastase, viz., that the precipitated substance is not free from mineral matter (ash). The amount of ash decreases if the diastase is dissolved in water and precipitated by alcohol, but at the same time it has lost some of its strength, so that we arrive at this somewhat paradoxical conclusion, that the purer the diastase, the less active it is. Purification, instead of increasing, decreases the characteristic property of diastase, viz., the power of saccharifying starch.

Lintner made the activity of his preparation the standard (= 100) or basis of comparison.

Osborne, however, as previously mentioned, succeeded in obtaining preparations of malt diastase, possessing much higher diastatic power, by precipitating the diastase from cold-water extracts by means of ammonium sulphate, and by repeated solution in water and dialysis into alcohol whereby he succeeded in obtaining diastase practically free from ash and having a composition approximating very closely to vegetable albumin. The preparation had a diastatic power of 600, that is, six times as great as that obtained by Lintner. Diastase prepared by the foregoing processes is a white or pale yellow coloured substance readily soluble in cold water, giving slightly opalescent solutions. Its composition has been determined by various workers, but considerable difference is shown in the different preparations obtained, particularly in regard to the nitrogen percentage which may be taken as a guide to the relative purity of the respective samples.

Owing to the great diffusibility of diastase contained in malt, it has been a very easy task for chemists to prepare it from cold-water extracts of malt, and they have been enabled in this manner to make a relative determination of the amount of diastase contained in different malts, and to compare them from

that point of view. The extract of malt prepared in this way has been generally employed for the study of diastatic action, and the researches of Brown and Heron, followed by those of Brown and Morris, have established the laws to which this action is submitted, to the great benefit of the brewing trade.

These laws are so well known that it is only necessary to *briefly* record them.

The maximum energy of diastase, in solution, is obtained at a temperature slightly below 140° F. (60° C.), immediately above this temperature it commences to lessen in activity. At 151° F. (66° C.) this is distinctly pronounced, whilst at 169° F. (76° C.) its action is almost entirely arrested, and at 177° F. (80·5° C.) its power is destroyed.

Although, however, solutions of diastase are extremely sensitive to the influence of heat, solid preparations carefully desiccated and free from moisture may be heated to 250° F. (121·1° C.) without their activity being destroyed. This different effect produced by heat upon the moist and dry substance fully explains the practical observation of the great loss of diastatic activity in malt during the first few hours upon the kiln, when the corns contain a large percentage of moisture, and the relatively slight influence of high-kiln temperatures when the malt is completely dry.

Fernback states that the action of diastase, as well as that of other enzymes, is, within certain limits, favoured by an increase of the temperature.

The amount of starch transformed by a given quantity of malt extract increases as the temperature rises. This point is very well illustrated by Ehrich[1]:—

Temperatures of Saccharification. °F.	°C.	Time employed for Saccharification. Minutes.
140	60	120
149	65	25–30
158	70	10
167	75	10

But there is another peculiarity of great importance in the practice of brewing which, of all enzymes, diastase alone shows. The amount of fermentable sugar varies with the temperature of saccharification. The lower the temperature the greater the amount of maltose. So that, by employing a definite temperature

[1] *Der Bierbrauer*, 1896, No. 7.

of saccharification, the brewer has in his hands the means of pro ducing with a given malt a wort of a given composition, which will, during the fermentation, yield a given attenuation.

We also find, in the researches of Brown and Morris, a third point of the greatest practical importance. When an extract of malt has been heated to a certain temperature, and is afterwards employed for the saccharification of starch paste at a *lower* temperature, the amount of maltose produced is the same as that which would have been produced at the high temperature to which the extract of malt had been previously heated. We learn from this observation that the greatest attention should be paid to the mashing temperature, as no change at all will be brought about by the addition of cold water, if the temperature has been raised too high at a certain period.

Science cannot at present give a satisfactory explanation of these facts. It was formerly supposed that saccharification was produced by two distinct enzymes, one transforming starch into maltose, the other producing dextrin. The former of these enzymes was supposed to be destroyed at a lower temperature than the latter, so that heating the malt extract restricted the enzyme-producing maltose to a certain extent, and the liquid obtained produced a wort containing less maltose than was furnished by the same extract unheated. Different attempts have been made, especially by Wijsman, to prove the existence of these two enzymes, but, up to the present, no convincing experiment has been published, and it seems that this hypothesis has to be abandoned.

In addition to these considerations, it must be stated that although we know what effect the action of heat has on diastase from a practical point of view, we have very little information on the way in which diastase is affected by heat. In a series of experiments, Fernback noticed that if a solution of pure diastase (after Lintner's process) be prepared and heated to 140° F. (60° C.) in a water bath, it will, in a very short time, after less than one hour, have lost a great part of its activity. This effect will be observed even if the solution be heated *in vacuo*, so that the possibility of an oxidation cannot be admitted, and is out of the question. This temperature is one of the lowest at which saccharification can practically operate, so that we are forced to this conclusion: that, in malt, diastase is accompanied by substances which confer on it a certain immunity—a certain resistance—against the destructive action of heat. It is nevertheless most probable that, during the saccharifying process, a

part of the diastase undergoes destruction, and from that consideration we understand the great practical utility of a fact which is most generally observed, viz., that malt contains an amount of diastase greatly superior to what is necessary for the transformation of the starch present in the grain.

The twofold function of diastase, namely, the liquefying and saccharifying, is only found in malt diastase. Kjeldahl showed, in 1879, that barley diastase has the saccharifying power, but not the liquefying. This latter power of malt diastase appears to be closely associated with its ability to slowly dissolve the outer cell membrane of the starch granule, hence if barley starch be digested in the cold with a solution of malt diastase, erosion or pitting of the starch granule occurs, an action which can be distinctly observed by microscopical examination. Effront has shown that, if malt or malt diastase is added to a cold-water extract of raw grain, the power of the diastase is increased; and that if malt or malt diastase is added to a raw-grain infusion at a temperature beyond that at which diastase is destroyed, liquefaction of the starch takes place, but no saccharification. Although diastase is capable, however, of exercising a liquefying power upon gelatinised starch even in the cold, its action upon ungelatinised starch is slow and negligible from a practical point, this being due to the resistance offered by the amylo-cellulose which constitutes the envelope of each starch granule. Hence, it is necessary, in order that the action of the diastase upon starch granules may be effective, to conduct the operation at temperatures favourable to the solution of the cellulose by the diastase.

It has been found that between 130° and 140° F. (54·4° and 60° C.) the hydrolysis of the cell wall is rapid and complete in the case of malt and specially prepared raw grain such as maize and rice flakes or grits, but that in the case of unprepared raw grain it is necessary to conduct the operation at temperatures more closely approaching the gelatinisation point of the starch of the particular cereal employed.

As previously mentioned, then, the saccharifying power of malt diastase is destroyed at a temperature of 177° F. (85·5° C.). The liquefying power, however, is not wholly destroyed even up to a temperature of 220° F. (104·4° C.), and this is, therefore, a point of considerable importance in the liquefaction of starch when carrying out raw-grain conversion in specially constructed vessels.

The nature of the medium in which diastase acts, whether acid or alkaline, also exerts a very important influence not only upon

the activity of the enzyme in respect to the amount of starch hydrolysed, but also upon the nature of the products formed. Its maximum action is exerted in neutral solutions, comparatively small quantities of free acid or alkali being extremely prejudicial.

Brown and Morris found that when only slightly alkaline (·005 per cent.) with barium hydrate, sodium carbonate, or sodium hydrate, its saccharifying power is very much restricted, and that further increase of alkalinity completely inhibited its power. The restrictive action of free acid is also well known.

Ling showed that diastatic action is completely arrested by the presence of ·007 per cent. hydrochloric acid and by alkalies. Other acids, even carbonic, in like manner exert a restrictive influence. On the other hand, the presence of acid salts, particularly the phosphates, appears to exert a favourable influence.

Effront has shown that diastatic action is favoured by the presence of small amounts of asparagin, ammonium acetate, ammonium chloride, alums of potash and soda, phosphoric acid, and of ammonium phosphate.

In the presence of 0·4 per cent. of asparagin, the amount of maltose calculated on the dry substance after one hour's action at 122° F. (50° C.) was found to be 58·2 per cent., whereas without asparagin it was only 16·4 per cent. Analogous effects were observed with the other bodies mentioned, and as the presence of some of these substances is not excluded, moreover, is certain, in the extract of malt, we must take them into account, and we are led to conclude that they play an important part in diastatic action.

A mash always shows an acid reaction, but this is not due to free acid, but rather, as has been explained by Fernback, Prior, and others, to the presence of acid phosphates pre-existing in the malt or formed by the interaction of the saline constituents of the mashing liquor and the potassium phosphates of the malt.

From the foregoing details it will be understood why many conflicting statements have been made by different observers and investigators as to the absolute energy of diastase. Payen and Persoz estimate that it is capable of converting 2000 times its own weight of starch into sugar.

Dubrunfaut, who pursued his investigations on this active agent up to the time of his death, alleges that malt contains 1 per cent., and that it possesses a power capable of converting 150,000 to 200,000 times its weight of starch. Roberts found pancreatic diastase to convert 40,000 times its weight of starch, and other distinguished observers state that malt contains only

·002 to ·003 per cent., and that its converting power is as stated by Payen and Persoz.

Although the converting power of diastase is very great, a small quantity being capable of saccharifying a very large amount of starch, this statement, as will have been seen, only applies when *time*, *temperature*, and *concentration* are taken into account. Hence when the time is limited, the converting power of the enzyme is also limited. In equal intervals of time, and under definite conditions, the quantity of starch converted is proportional to the amount of active enzyme present.

This is what is known as Kjeldahl's "law of proportionality," and was expressed by him in 1879 as follows:—"*When equal volumes of two diastatic solutions are allowed to act upon solutions of soluble starch under identical conditions of time, temperature, and concentration, then the cupric reducing power of the two solutions, taken at any given time, is a measure of the relative transforming powers of the diastatic solutions, providing the cupric reducing power when calculated as percentage of dextrose is not allowed to exceed K* 25 *to* 30." In other words, if the amount of maltose formed does not exceed 40 to 50 per cent. of the starch originally taken, the relative activities of the two solutions can be determined from the amounts of maltose formed in a given time, since, under such conditions, the formation of maltose is proportional to the amount of diastase present. In the mashing process the amount of maltose formed is, however, not necessarily proportional to the diastatic powers of the respective malts, since, in this case, the diastase is present in excess, and, further, the products of hydrolysis exceed the proportion above stated.

In 1886 Lintner elaborated a more accurate method for the estimation of the diastatic power of malt, such method being based on the same law—"the law of proportionality"—and being the one now considered as the most satisfactory. Details for carrying out the estimation are given hereafter under "Diastatic Power" in malt analysis.

During the conversion of soluble starch by diastase, the products formed consist of free maltose and dextrin, and of intermediate bodies termed malto-dextrins or amylöins, which are compounds of maltose and dextrin.

Although such intermediate products are undoubtedly formed, the result of the action of diastase upon soluble starch can always be expressed in terms of free maltose and dextrin. This "*law of definite relation*," enunciated by Brown and Morris, is as follows:—"*The products of a starch transformation can always*

be expressed in the terms of maltose, having an optical activity of $[\alpha]_{j\,3\cdot86} = 150$, *and a cupric reducing power* $K_{3\cdot86} = 61$, *and of dextrin having an optical activity of* $[\alpha]_{j\,3\cdot86} = 216$, *and no reducing power.*"

Under normal conditions diastase has no further action upon the maltose and dextrin first formed, but the malto-dextrins are capable of further hydrolysis, being wholly converted by continued digestion into maltose, the dextrin content of the malto-dextrin thus behaving differently to the free or stable dextrin.

Glucase or Maltase.

The hydrolysis of carbohydrate bodies to the simple hexose sugar glucose is performed by the group of enzymes termed glucase or maltase, these designations being to a great extent synonymous according to the different systems of nomenclature adopted by different workers. Of the two glucase appears the better term to employ, since it points to an enzyme capable of acting upon carbohydrate substances producing glucose, whereas the designation maltase appears to restrict the term to enzymes capable of hydrolysing maltose only.

As will be shown later, the enzyme of yeast, which is capable of decomposing the disaccharide maltose into the simple hexose sugar glucose preliminary to fermentation, has also an action upon dextrins, malto-dextrins, and, under certain conditions, hydrolyses starch; hence it is preferable to employ the term glucase for this enzyme rather than that of maltase, which was at first adopted, and is now sometimes employed.

The glucases differ in their nature and properties according to the source from which they are derived, those which have received the most attention being derived from maize and yeast respectively.

Musculus and Gruber were the first to mention the existence of glucose as being produced by the action of diastase on starch in addition to the main product maltose. This statement, therefore, is tantamount to showing that glucase exists in malt, but opinions of different investigators are divided as to whether it does or does not exist in malt; the balance of evidence so far adduced, however, is in favour of its existence in certain classes of malt at any rate. Apart from malt, Cuisenier, in 1855, proved the existence of glucase in maize, and commercially worked an industrial process for the production of glucose from maize.

Mering was the first to suggest that yeast contained a "ferment"

capable of converting maltose into glucose before alcoholic fermentation, and Bourquelot shortly afterwards proved that maltose is hydrolysed before fermentation is started, by acting upon solutions of maltose with yeast in the presence of chloroform, whereby the action of yeast cells is suspended.

More recent work, viz., that of Emil Fischer, has resulted in substantiating Bourquelot's statement that yeast contains an enzyme which hydrolyses maltose to glucose preliminary to fermentation, the presence of the glucose being identified by his now well-known osazone reaction with phenyl-hydrazine. Fischer's investigations in these respects have since been repeated and confirmed by Morris, who extracted the active enzyme from yeast cells, quickly dried upon porous plates.

Besides the glucase of maize and yeast, the enzyme is widely distributed in nature, and is frequently associated with the diastatic enzymes. Bourquelot identified it in many plants and nearly all moulds. It is not found, however, in the *lactose* or *kephir* yeasts, *S. marcianus* or *S. apiculatus.*

In the majority of instances it is looked upon as a starvation phenomenon, secreted by the living cell in proportion as it is deprived of the presence of the simple hexose sugars, such as glucose, which alone appear to be directly assimilable. Thus many moulds which ordinarily contain glucase do not secrete the enzyme at all if continuously supplied with saccharose or glucose, but if their supply of these sugars is cut off and maltose substituted, then the secretion of the enzyme, glucase, is at once stimulated in the plant cell by its deprivation of suitable food.

Glucase is found associated with invertase and diastase in *Aspergillus oryzæ*, and it has been shown by Atkinson that the final product of the action of this enzyme on gelatinised starch is glucose and not maltose.

Glucases have been found in the saliva, secretions of the intestines and the pancreas, and in various organs of animals.

The methods adopted for extracting glucase are similar to those employed in the extraction of diastase and invertase, the extraction being accomplished by suitable solvents and precipitation by alcohol. In the case of yeast, which in the fresh state yields no glucase to water, the yeast must first be dried quickly upon porous plates at low temperature, and the resulting mass, after pulverising, digested with water at 86° – 95° F. (30° – 35° C.), or by extraction with 1 per cent. sodium hydrate, and subsequent precipitation.

To study the action of the glucase of yeast upon maltose, fresh

yeast is suspended in a solution of maltose. If the fermentative action of the yeast be inhibited by the addition of chloroform, toluene, or thymol, in the course of a few hours the progress of the hydrolysis of the maltose can be followed by the lowering of the opticity of the solution and the increase of its cupric-reducing power.

The glucase prepared from maize is of a light, friable character, brownish in colour, dissolving in water with difficulty, and precipitated from its solutions by alcohol. Aqueous solutions of glucase of different origin quickly lose their activity, and their action is weakened by the presence of alcohol, chloroform, and antiseptics. The enzyme acts most energetically upon maltose, but most glucases are capable of acting upon dextrin and maltodextrins, and some are capable of hydrolysing starch. As a rule the glucase of cereals hydrolyse maltose readily, but acts with difficulty upon starch, whilst on the other hand the glucase of moulds acts more vigorously upon starch than upon maltose. The presence of a slight acidity is favourable to the action of the glucases, but is entirely arrested when the acidity of the medium is equal to or above ·2 per cent.

The optimum temperature and that at which the enzyme is destroyed varies with its origin as follows:—

Origin.	Optimum Temperature.		Destroyed at	
	°F.	°C.	°F.	°C.
Yeast . . .	120·2	49	131·0	55
Penicillium glaucum .	113·0	45	158·0	70
Eurotiopsis gayoni .	122·0	50	131·0	55
Aspergillus niger .	140·0	60	176·0	80
Maize . . .	140·0	60	158·0	70

Cytase.

In their epoch-making paper, "Researches on the Germination of some of the Gramineæ," Brown and Morris published an account of the existence of an enzyme produced during the germination of barley to which they gave the name cytase.

In order to thoroughly understand the action of this enzyme, it is necessary to be first familiar with the structure of the barleycorn and the morphological changes occurring during germination.

As before stated, under Diastase, plants possess three distinct diastases, called respectively *secretion diastase*, *translocation diastase*, and *cytase*. The first named is that present in germinating seeds; the second is the diastase of ungerminated seeds and

other plant organs; whilst cytase is an enzyme which possesses the power of dissolving the cell walls.

The distinguishing feature between secretion and translocation diastase is the inability of the latter to exercise any solvent action on ungelatinised starch granules.

In the barleycorn the columnar cells (termed the epithelium, forming the membrane intervening between the endosperm and embryo) secrete the diastase necessary for the resolution of the reserve material stored up in the endosperm, performing a duty analogous to that of the alimentary canal and of the walls of the stomach, which secrete the enzymes necessary for the proper digestion and assimilation of food by animals.

During malting it was formerly considered that diastase was the immediate cause of that mealiness and friability which indicates the modification of malt; or, in other words, that the starch of the endosperm was gradually modified by the action solely of secretion diastase. It is now shown, however, that before this diastatic action can proceed, it is first necessary for the cell walls to be dissolved, and the enzyme which performs this dissolving action is cytase.

Cytase is the agent which attacks the cellulosic tissue of the endosperm cells, breaking it down and causing its *complete dissolution.*[1] Following this there is an erosion of the starch granules by secretion diastase, but this action on the starch granules never takes place so long as the walls of the cells containing them are intact.

The two enzymes—diastase and cytase—are quite distinct, the action of cytase on the cell walls of the endosperm always preceding that of the diastase on the starch granules. The production of mealiness or friability in the content of the endosperm is therefore, according to Brown and Morris, "coterminous with the dissolution of the cell wall," and is entirely independent of the disintegration of the starch granules.

In their endeavour to locate cytase in the barleycorn, Brown and Morris state that the elaboration of the enzyme, as well as the diastases, is carried out by the columnar cells forming the epithelium dividing the scutellum of the embryo from the endosperm; and that its first action could be traced upon the depleted cells adjacent to the embryo, and thence proceeding slowly

[1] It appears to be an open question as to whether the cell walls are entirely or only partially dissolved by cytase, but apart from this it is sufficient that cytase opens the door sufficiently wide, so to speak, to render the starch of the endosperm permeable to the action of secretion diastase.

throughout the corn. Tangl and Haberlandt were able to show, however, that the secretion of cytase is not due to the epithelial cells alone, but that a triple row of cells surrounding the endosperm, termed *aleurone cells*, exercise a marked influence upon the progress of the dissolution of the cell wall, and this view was afterwards accepted by Brown and Escombe.

Sachs was the first to demonstrate the existence of a special enzyme capable of attacking the less resisting forms of cellulose during germination of the date seed, and later Green endeavoured to isolate the enzyme. Brown and Morris were the first, however, to isolate cytase from malt, and this they accomplished by making infusions of air-dried malt, precipitating the enzyme with alcohol, and carefully drying the precipitate *in vacuo*. This preparation was found to exhibit marked cyto-hydrolytic action (cellulose dissolving power). Its action upon cellulosic tissue is, however, more or less restricted to the cell walls of the endosperm of the seeds from which the enzyme has been derived, or similar tissues from seeds belonging to the same material order. Thus the cytase derived from germinated barley acts readily upon the cellular tissues of the endosperm of the Gramineæ, but is less active towards similar tissues from other groups; and it appears to be a general rule that the cytases are most active upon the cell walls of the particular seed in which the enzyme has been elaborated.

Cytase is not capable of acting upon all forms of cellulose, this term being used to embrace the members of a general group of substances, forming the fabric of plant structures, similar in chemical composition but differing in certain chemical and physical characteristics. Thus the cellulose material forming parenchymatous tissue, as found in the cellular membranes of the barleycorn, is less resistant to chemical and enzyme action than the lignified tissues of cellulose constituting the husk. As will have already been seen under Cellulose, the former kind is termed *hemi-cellulose* in order to distinguish it from the more stable and resistant forms which are unacted upon.

The products of the action of cytase are generally understood to be substances of a gummy nature, but have not yet been identified; whatever the nature of such products may be, it is generally considered that they serve as food for the embyro during the early stages of germination.

Güss considers the hemi-cellulose of barley to be of the nature of arabinoxylan, yielding, as the result of cyto-hydrolysis, the pentoses arabinose and zylose, and similar substances have been

shown to exist in cold-water extract of malt, Morris indicating that the unfermentable reducing residue of beer may in some measure be attributed to the products of cytase hydrolysis during germination.

Cytase is very sensitive to the action of heat. The activity of a cold-water infusion of air-dried malt is greatly weakened in its action, so far as cellulosic tissues are concerned, if heated to 122° F. (50° C.), and this power is completely destroyed if heated for a short time to 140° F. (60° C.), although the amylo-hydrolytic power of diastase is still energetic in its liquefying and saccharifying action upon starch granules beyond these temperatures. Owing to this ready destruction of the enzyme, it will be understood that only green malt, dried at a low temperature, contains active cytase, ordinary malt being devoid of it owing to its complete destruction during kiln-drying.

Invertase.

Invertase was discovered in 1830 by Doebereiner and Mitscherlich, who described it as a "soluble material" extractable from yeast when the latter substance is mixed with water. The soluble material liquefies cane-sugar and produces inversion in it by causing it to take up the elements of water.

In 1860 Berthelot discovered that invertase could be precipitated, by means of alcohol, from its solution without losing its activity, and since this date numerous experimental data, showing means by which the ferment can be extracted from yeast and its properties when extracted, have been produced by Béchamp, Donath, Seyler, Gunning, Tulkowsky, König, Kjeldahl, Mayer, Barth, and Müller. In all their experiments, however, no notice was taken of the age of the yeast or its condition as regards health or disease, and this seems the more strange considering that long previously (1835) Schwann had shown Leuwenhoek's globular yeast particles to be alive. All of any considerable importance that was pointed out in this direction was that dead cells parted with their invertase more freely than living cells, no mention being made of the further fact that healthy yeast yields none of its invertase or hydrolysing agent or enzyme to water in which it is washed.

The widespread occurrence of cane-sugar or saccharose in nature is well known, but the *bioses*, to which group cane-sugar belongs, are not directly fermentable or immediately available as plant food, but require to be split up into the simple *hexose* sugars, dextrose and levulose, before either fermentation or assimilation

by the living cell can take place. Hence it is that nature provides the transforming enzyme, and that cane-sugar in nature is accompanied by the existence of invertase in the saps and juices of plant cells which depend for their nourishment upon the assimilation of the sugar.

Invertase plays an important part among the higher plants in as much as by its aid the cane-sugar carried by the sap is rendered available for building up the cell fabric. It is found in all parts of the Gramineæ; C. O'Sullivan demonstrated its presence in the roots, stalks, and leaves of wheat and maize, and others have noted its presence in the leaves of many plants belonging to different natural orders. Its presence in green malt is undoubted, and according to Brown and Morris, Kjeldahl and others, it is present in kilned malt, not being wholly destroyed during kilning. In animals, invertase has been located in some of the organs, its presence in the intestinal secretions being first discovered by Bernard.

Invertase is invariably present in yeast cells, usually associated with other enzymes such as glucase and zymase. It is not, however, present in all yeasts, for Hansen has shown that *Saccharomyces apiculatus*, the yeast found on many fruits, is devoid of it. Invertase has been found in many of the mould fungi, among which the genus *Aspergillus* may be specially mentioned, the invertase of *Aspergillus niger*, *Aspergillus oryzæ*, or taka ferment having been closely investigated. Invertase is also secreted by *Mucor racemosus*, *Penicillium glaucum*, and *Penicillium Duclauxii*. It also occurs in many species of *Fusarium*, and has been shown to be present in *Monila candida*. Its occurrence in this organism is of special interest from the fact that, although it readily transforms cane-sugar, an active enzyme cannot be prepared from it by the usual methods, the investigations of Fischer and others having demonstrated that, unlike the invertase of most organisms, the invertase secreted by *Monila candida* is insoluble.

Invertase has also been shown to be secreted by many bacteria, among which *B. mesentericus vulgatus*, *B. megatherium*, and the *Cholera vibrio* may be mentioned. It also occurs in *Leuconostoc mesenteröides*, the "frog spawn" of beet-sugar juices. J. O'Sullivan states:—

"1. Healthy yeast yields none of its invertase or hydrolytic power to water in which it is washed.

"2. When healthy yeast is placed in contact with cane-sugar solution, the hydrolysis that takes place is solely an action under the immediate influence of the plasma of the cell, and no invertase

leaves the yeast cell during the time the hydrolysis is being effected.

"3. The hydrolytic action of yeast on cane-sugar takes place without increase of yeast, and there is no alcohol formed. The power which yeast possesses of producing alcoholic fermentation is not altered in any way by the yeast having first hydrolysed sugar-cane, and this is the case whether the hydrolytic action is brought about in the first instance in the presence of air or carbon-dioxide."

C. O'Sullivan and Tompson point out as follows:—

"1. When yeast is placed in cold water and allowed to remain for a few hours, what is known as 'osmosis' takes place, that is to say, a soluble albuminous substance contained in the torpid and dead cells, being extremely soluble in water, diffuses through the cell membrane of the yeast and passes into the exterior liquid.

"2. If sound brewers' yeast be pressed and placed in a large wide-mouthed vessel covered with a filter paper to prevent access of dust, and allowed to stand at the ordinary temperature for a month or two, or at a temperature of 64·4°–68·0° F. (18°–20° C.) for 7–14 days, during this time auto-fermentation of the yeast takes place, and the mass liquefies, yielding a thick yellow liquid of not unpleasant smell. If this liquid is then filtered by the aid of a filter pump, a clear yellow liquid is obtained, having a specific gravity of 1075°–1080°. The filtrate has an inverting power equal to 30 per cent. of the original yeast. It will remain for a long time unaltered, excepting that the colour darkens; and if exposed to the air, it may slowly become covered with mould. If spirit be added to the yeast liquid until the mixture contains 47 per cent. of alcohol, the whole of the invertase separates with only a slight loss of power.

"This precipitated invertase may be washed with spirit of the same strength, and then the residue either dehydrated with strong alcohol and dried *in vacuo*, or else it may be extracted by means of 10 to 20 per cent. alcohol and then filtered. The filtrate contains the invertase. On one occasion it was determined what the loss involved by this process amounted to, and it was found that 87·7 per cent. of the invertase of the yeast liquor was present in the filtrate."

C. O'Sullivan and Tompson have not, up the present, succeeded in further purifying invertase preparations carefully made in this manner, as the slightest attempt at purification destroys the invertase. They have, however, succeeded in preparing the enzyme almost free from ash.

Invertase prepared by the foregoing process is a fairly white powder, which soon becomes yellowish on exposure to air and light. It is soluble in water, giving clear solutions which are not coagulated on heating. Solutions of invertase are dextro-rotatory, and, according to C. O'Sullivan, the specific rotatory power of *pure* invertase is $[\alpha]_j = +80°$. Invertase is readily precipitated from its solutions by the addition of alcohol, and is completely insoluble in alcohol of specific gravity ·940°. The precipitates are of a semi-syrupy nature, and usually contain inorganic salts precipitated from the solutions by alcohol, although a considerable quantity of these inorganic bodies can be eliminated by washing the precipitate with alcohol.

Invertase can be separated from solutions by filtration through porcelain or by parchment in a dialysing apparatus. It is difficult to keep it either in solution or as a dry preparation without loss of power, this being probably due to oxidation in the presence of light. It is extremely sensitive to temperature above the normal or optimum when in solution, but when perfectly desiccated will withstand the temperature of the boiling-point without appreciable loss of power.

Little is known of the constitution of invertase; most authorities concur that it is not an albuminoid. It gives no coloration with Millon's reagent in the cold, but on heating, a pink coloration is produced. It does not reduce Fehling's solution.

O'Sullivan considers invertase, as separated from yeast, to be a mixture of yeast albuminoid and a carbohydrate. As the invertase approaches the pure state it becomes a very unstable body, the purification resulting in the formation of decomposition products designated the *Invertan* series, which may be expressed in terms of a carbohydrate of the formula:—

Carbon, 43·22, hydrogen, 6·28, oxygen, 50·40, and an albuminoid (yeast albuminoid)—

Carbon, 54·33, hydrogen, 7·50, nitrogen, 14·88.

Seven members of the *Invertan* series were examined and their properties described. According to O'Sullivan the proportion of carbohydrate to yeast albuminoid varies from ·74 part in *a Invertan*, the first member of the series, to 12·84 parts in *n Invertan*. All the members of the group are devoid of inverting power with the exception of the second member of the *b Invertan*, this constituting the true enzyme invertase.

The action of invertase upon cane-sugar is one of simple hydrolysis, and the change, which is precisely the same as that

effected by acids, consists in the assimilation of water by the cane-sugar molecule, followed by its separation into the two different kinds of sugar—dextrose and levulose—which though of very different properties have the same formula :—

$$\underset{\text{Cane-sugar.}}{C_{12}H_{22}O_{11}} + \underset{\text{Water.}}{OH_2} = \underbrace{\underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Levulose.}}{C_6H_{12}O_6}}_{\text{Invert-sugar.}}.$$

Like most enzymes, the action of invertase is influenced by concentration, temperature, and the nature of the medium; the amount of inversion being governed by the quantity of invertase present and the time its action is allowed to continue.

The most favourable concentration for sugar solutions is between 20 and 30 per cent. of sugar, a decline in the speed of inversion taking place when the solutions are dilute or in saturated solutions above 40 per cent. The rapidity of inversion is greatly influenced by the temperature. At low temperatures the inversion proceeds slowly, rapidly increasing with every increment of temperature until the optimum of 131°–140° F. (55°–60° C.) is reached. The optimum temperature is not a constant one, but varies according to the source from which the invertase is derived. The invertase of top fermentation yeast acts best at a temperature of 131°–140° F. (55°–60° C.), whilst the invertase from bottom fermentation yeast exhibits its maximum effect between 86°–95° F. (30°–35° C.). At temperatures above 140° F. (60° C.) the activity declines, and the enzyme is weakened by exposure to the elevated temperature until at 167° F. (75° C.) it is totally destroyed.

The activity of invertase is at a maximum when acid is present in normal amount, this, according to O'Sullivan being, for temperatures of 131°–150° F. (55°–65·5° C.), 1·25 parts SO_3 per 100,000 (= ·0016 per cent. H_2SO_4).

It is, however, extremely sensitive to any increase of acidity. The presence of alkali is absolutely destructive to its action, and the presence of alcohol is distinctly prejudicial, the decrease of activity being proportional to the amount of alcohol present. The action of invertase upon cane-sugar is rapid in regard to speed and enormous as to amount. O'Sullivan illustrates these points by two striking experiments :—

A preparation of invertase reduced the optical activity of a solution of cane-sugar to the zero point $[\alpha]_D = 0$ in 25·1 minutes, the sugar being present in the proportion of 100 to 1 of invertase added. This is equivalent to the inversion of 74 times its own

weight of cane-sugar in 25·1 minutes. By adding a weighed quantity of invertase to a large bulk of cane-sugar, and digesting at a temperature of 122°–129·2° F. (50°–54° C.) for a fortnight, it was found that an inversion equal to 100,000 times its weight of invertase added had taken place, and that the inverting action was still slowly progressing.

Zymase.

In 1897 the scientific world was startled by the discovery by Dr E. Buchner of an enzyme extractable from yeast capable of fermenting solutions of cane-sugar. The idea was at first looked upon as incredible, owing to the long-cherished famous dictum of Louis Pasteur, viz., that "Fermentation is the physiological effect of the life-action of yeast and other cells." Buchner, however, very quickly proved his point, having succeeded in extracting from yeast an active liquid capable of effecting the decomposition of sugar without the direct agency of living cells, and established beyond question that the production of alcohol by yeast—fermentation—does not differ essentially from other zymolytic decompositions, but is analogous to the inversion of cane-sugar by invertase, and the hydrolysis of maltose by yeast glucase. Buchner obtained the active alcoholic enzyme as follows:—

Fresh yeast is first subjected to a pressure of 750 lbs. to the square inch, in order to free it from adherent liquid. It is then mixed with an equal weight of quartz-sand and a fifth of its weight of kieselghur[1], and placed in a grinding mill driven by a motor, and ground until the mass, at first pulverulent, becomes moist and adheres together in balls. The object of this grinding is to rupture the walls of the yeast cells, and to permit their protoplasmic contents to escape. The doughy mass is then enveloped in a cloth and placed in a hydraulic press, where it is slowly and gradually submitted to pressure until at last a pressure of between 3 and 3½ tons to the square inch is reached. After about two hours the cake is removed from the press, broken up, and moistened with water. It is then pressed as before. In this way a mass of yeast yields about half its weight of yeast extract, rather less than three-tenths of this extract consisting of the water added to moisten the yeast cake. The yeast extract, as it drops from the press, is allowed to pass through a funnel, provided with a filter paper, into a vessel which is kept in ice, in order to prevent decomposition of the extract as much as possible

[1] A fine, earthy powder which consists of the siliceous coverings of diatoms.

during its preparation. A microscopic examination of the yeast cake after the second pressing shows that only about 4 per cent. of yeast cells remain intact. The extract obtained in this way is a clear or slightly opalescent liquid, with a pleasant yeasty odour, its specific gravity being about 1041·6. On *boiling*, a considerable amount of coagulum forms, so much so that the liquid is converted into an almost solid mass. But the most remarkable property it possesses is the power of inducing fermentation.

When equal volumes of the extract of a concentrated solution of sugar-cane are mixed, or if powdered sugar-cane is dissolved in the extract, in from 15 minutes to an hour a regular evolution of carbon-dioxide commences, and this continues for several days. Saturation of the mixture with chloroform does not arrest the action, though it is a well-known fact that this substance completely stops fermentation when conducted with ordinary living yeast. The fermentative power of the extract is not destroyed when the extract is passed through a Berkefeld filter, and this treatment would certainly remove from it all living organisms.

The yeast extract gradually loses its fermentative power when kept at the temperature of an ice chamber, much more rapidly at higher temperatures. It has been pretty conclusively shown that this is due to the destruction of the zymase by another enzyme of a proteolytic nature present in the extract. Living yeast is unable to ferment solutions of cane-sugar of 50 to 60 per cent. strength, or solutions to which large quantities of glycerine have been added; zymase ferments these readily. Like most other enzymes, zymase can be precipitated by alcohol, but on subsequent solution in water it is found to have lost very much of its activity. It may be obtained, however, in the dry state, with little or no loss of activity, in the following manner:—

500 c.c. of fresh yeast extract are evaporated, in a vacuum apparatus, at a temperature of 68°–77° F. (20°–25° C.) until of a syrupy consistency; the syrup is then spread in thin layers on glass plates and dried at a temperature of 93°–95° F. (34°–35° C.) either in a vacuum or in the air, since it is found that, when the above-mentioned degree of concentration has been arrived at, air has no longer any effect upon the zymase. The dried extract is then scraped off the plates, reduced to a powder, and brought to a state of absolute dryness by being placed in a desiccator over sulphuric acid. The 500 c.c. of yeast extract is found to yield about 70 grams of this yeast powder, which has a pleasant yeasty odour, and in appearance somewhat resembles dried white of egg. If the 70 grams of yeast powder so obtained

are dissolved in 500 c.c. of water, that is, made up to the same strength as the original yeast extract from which it was prepared, a solution is obtained which possesses nearly the same fermentative power as the original yeast extract.

In the dried form the fermentative power of the enzyme can be preserved for a longer period than in the liquid state, some preparations retaining their activity for 20 days, whilst those concentrated *in vacuo* preserved their full activity for over two months.

Buchner further demonstrates that the yeast cell can be so dried as to destroy its vitality without impairing the activity of the zymase, which can still exert its fermentative function *in situ* within the cell membrane. The yeast is first well washed with water, and after removing extraneous moisture as much as possible by pressure, it can be perfectly dried in two or three days if spread out in thin layers, A yellow powder is obtained which, on heating to 212° F. (100° C.) for 5 to 6 hours, becomes completely sterile, the vitality of the cells being wholly destroyed and no longer capable of developing in nutrient solutions. That the zymase is not destroyed by this procedure is evidenced by the fact that, when the powder is placed in a saccharine solution, fermentation is set up.

Yeast extract, representing the sap or juice of the cell, contains, besides zymase, those other enzymes known to be secreted by the living cell, viz., invertase and glucase, in addition to which a proteolytic enzyme and an oxydase have been noted. The presence of an amylase, however, does not appear to have been definitely established. Hence the zymase is capable of fermenting those sugars which are ordinarily decomposed by the living cell, whether directly as dextrose and levulose, or fermentable only after hydrolysis, such as saccharose, maltose, etc., which require the presence of specific enzymes. It has been found that maltose, saccharose, dextrose, and levulose are equally rapidly fermented by zymase. In the case of the two last-named sugars, however, this is not the case when fermented by the living cell, the greater diffusibility of dextrose through the cell membrane causing the fermentation of this sugar to proceed more quickly than that of levulose; in fact a solution of invert-sugar undergoing fermentation increases in lævo-rotatory power. With the expressed yeast extract (zymase) this phenomenon does not occur, both dextrose and levulose being decomposed uniformly. Of other carbohydrates, raffinose, galactose, and glycogen are very slowly attacked, whilst lactose and arabinose are not fermented

by it; in fact only those carbohydrates which are readily hydrolysed by the enzymes of ordinary yeast with the formation of simple hexose sugars are capable of fermentation by yeast.

Buchner, studying the specific action of zymase, found that the relative proportion of CO_2 to alcohol produced by the enzyme during fermentation, does not wholly agree with the ratio obtainable by living-cell fermentation, the amount of alcohol being usually in excess. In one experiment the quantities found were $CO_2 = 6{\cdot}7$ grams, alcohol 7·72 grams, an excess of ·72 grams alcohol.

Macfadyen and Morris also showed that the amounts of alcohol and carbon-dioxide produced were less than should be yielded by the amount of saccharose which disappears, so that it is evident that the decomposition of sugar by zymase, like the action of the living cell, cannot be a simple decomposition such as can be expressed by the equation of Gay-Lussac—

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_6O.$$

It is more than probable, therefore, that complex combinations between the enzyme and the carbohydrate, followed by decomposition, take place before the final products, alcohol and carbon-dioxide, result. In cell fermentation, too, secondary products, glycerol and succinic acid, are formed; and the investigations of Buchner show that these products are also formed by the action of zymase, although in much smaller amount.

The fermentative power of zymase was found by Lange to increase with the ratio of nitrogen, this fact being in accordance with the conclusions of Hayduck, who showed this to be the case with living yeast. Zymase appears to closely resemble albumin, but it differs in many respects from invertase. It is in some of its properties analogous to the enzyme of *Monila candida*, particularly in the extreme difficulty with which it diffuses, if at all, through cell membranes or parchment. Although the action of zymase is not impeded by the presence of antiseptics which are inimical to yeast growth, thus behaving as a true enzyme, it is extremely sensitive to the presence of excessive amounts of inorganic substances. Neutral salts in small quantity appear to favour the action, but if they are increased beyond 1·5 per cent., the action is completely arrested. Acids and alkalies are both unfavourable, but in minute quantities appear to stimulate it. Phosphates, particularly acid phosphates, in small proportion, exercise a stimulating effect. Fermentation is diminished by the addition of 15 per cent. alcohol, whilst the

presence of 20 per cent. completely arrests it, the enzyme being practically precipitated.

Here then we have the most conclusive evidence that *Buchner's yeast extract contains an enzyme which is the cause of alcoholic fermentation*, and as with other enzymes it has not been found possible to obtain zymase in a state of purity, no attempt has been made to ascertain its true chemical composition.

PART IV.

INDICATORS USED IN ALKALIMETRY.

Litmus Solution and Papers.

1. **Litmus Solution.**—It is customary, in testing both acids and alkalies, to employ a solution or papers of litmus. The solution may be prepared as follows:—

Weigh about 10 grams of solid litmus, reduce to powder, and boil with alcohol of about 80 per cent. Let stand for some time, then pour off the alcohol, it being no longer required for the preparation. Colouring matter, which is a hindrance to the proper reaction, is thus removed. Now digest the litmus repeatedly with cold distilled water till all soluble colour is extracted, let the mixed washings settle clear, decant, and add to them a few drops of concentrated sulphuric acid until quite red, then heat to boiling; this will decompose the alkaline carbonates and convert them into sulphates. Now cautiously add baryta water until the colour is restored to blue or violet, let the baric sulphate settle perfectly, and decant into a bottle for use. Litmus solution so prepared is very sensitive to dilute acids and alkalies; with the slightest excess of oxalic, sulphuric, hydrochloric, or nitric acids it gives a pink-red, and with caustic soda or potash, a blue colour; with ammonia or the bicarbonated alkalies it retains its violet colour, and the same with most of the neutral salts of the weak acids, such as sodium or ammonium acetate or borax. Free carbonic acid interferes considerably with the production of the blue colour, and its interference in titrating acid solutions with alkaline carbonates can only be got rid of by boiling the liquid during the operation, in order to displace the gas from the ammonia. If this is not done, it is easy to overstep the exact point of neutrality in endeavouring to produce the blue colour.

The same difficulty is also found in obtaining the pink red when acids are used for titrating alkaline carbonates, hence the great value of the caustic alkaline solutions free from carbonic acid in acidimetry.

Litmus papers are simply made by dipping strips of unglazed paper in the solution and drying them; if required red, the liquid is slightly acidified.

Neither the solution nor papers should be used by gaslight, nor, as stated, for testing any solution containing free CO_2.

2. **Methyl-orange** (a salt of sulpho-benzene-azo-dimethyl-amine).

Solution.—One gram in a litre of distilled water.

Used for titrating mineral acids in the cold: very useful for ammonia and its salts. Inapplicable for hot liquids and organic acids.

Special Properties.—Its indifference to CO_2 and SH_2 in the cold.

Colour Reaction.—Pink, with excess of acids. Faint yellow, with excess of alkali.

3. **Phenol-phthalein** ($C_{20}H_{14}O_4$).

Solution.—One gram in a litre of 50 per cent. alcohol.

Used for determining caustic fixed alkalies in presence of the carbonates. Gives no colour with the bicarbonates. Absolutely useless for the titration of free ammonia and its compounds, or for the fixed alkalies when salts of ammonia are present.

Special Properties.—Unlike methyl-orange, this indicator is specially useful in titrating all varieties of organic acids—oxalic, acetic, citric, tartaric, etc.

Colour Reaction.—Purple-red in alkaline solutions, rendered colourless by excess of acid.

4. **Cochineal Solution.**

Solution.—Digest one part of powdered cochineal with ten parts of 25 per cent. alcohol.

This indicator is not very much modified in colour by CO_2, and can be used by gaslight. Most useful in titrating solutions of the alkalies and alkaline earths. Inapplicable in presence of Fe or Al compounds or acetates.

Colour Reaction.—Yellowish-red turned violet by alkalies: mineral acids restore the original colour.

5. **Phenacetolin.**

Solution.—Two grams in a litre of alcohol.

Special Uses.—To estimate KHO or NaHO in the presence of K_2CO_3 or Na_2CO_3, or CaO in the presence of $CaCO_3$.

Colour Reaction.—

With NH_3 and normal alkaline carbonates—dark pink.

" bicarbonates—intense pink.

" mineral acids—golden-yellow.

6. **Rosolic Acid** ($C_{20}H_{16}O_3$).

Solution.—Two grams in a litre of 50 per cent. alcohol. This indicator is excellent for use with all the mineral acids, but unreliable for the organic acids.

Colour Reaction.—Pale yellow, unaffected by acids, turned violet-red by alkalies.

7. **Iodine Solution.**—One gram iodine and 1 gram potassic iodide in a litre of distilled water; or 1 per cent. solution of decinormal iodine.

Used for detecting starch in mashes, worts, etc., in the cold; inapplicable for hot liquids.

Colour Reaction.—Blue or violet with starch, reddish-brown with erythro dextrins.

8. **Ferrous Thiocyanate Solution.**—One gram of ferrous ammonium sulphate and the same quantity of ammonium thiocyanate are dissolved in 10 c.c. of distilled water at a temperature of about 120° F. (49° C.) and immediately cooled; 5 c.c. concentrated hydrochloric acid are then added. The solution so obtained has invariably a brownish-red colour, due to the presence of ferric salt, which latter must be reduced. For this purpose zinc dust is the most satisfactory reagent to employ, and a mere trace is sufficient to decolorise the solution if pure reagents have been employed.

When kept for some hours, the indicator develops the red coloration by atmospheric oxidation. It may, however, be decolorised by the addition of a further quantity of zinc dust, but its delicacy is decreased after it has been decolorised several times. For practical purposes the indicator may be too delicate, and it is therefore best to prepare it the day before it is required for use, as it gives the best results after the second decolorisation.

The indicator is employed for ascertaining the reduction of the copper in Fehling's test.

PART V.

PREPARATION OF STANDARD AND OTHER SOLUTIONS.

Standard Solutions.

When analysis by measure first came into use, the test solutions were generally prepared so that each substance to be tested had its own special reagent, and the strength of the standard solution was so calculated as to give the result in percentages. Consequently, in alkalimetry a distinct standard acid was used for soda, another for potash, a third for ammonia, and so on, necessitating a great variety of standard solutions.

Griffin and Ure were the first, however, to suggest the use of standard test solutions based on the atomic system, and, following in their steps, Mohr[1] worked out and verified many methods of analysis, which are of great value to all who concern themselves with scientific and especially technical chemistry.

Normal solutions as a general rule are prepared so that 1 litre at 60° F. (15·5° C.) shall contain the hydrogen equivalent of the active reagent weighed in grams ($H = 1$). Decinormal solutions are made one-tenth, and centinormal one-hundredth of this strength, and may be shortly designated as $\frac{N}{10}$ and $\frac{N}{100}$ solutions.

In the case of univalent substances such as silver, iodine, hydrochloric acid, sodium, etc., the equivalent and the atomic (or, in the case of salts, molecular) weights are identical; thus a normal solution of hydrochloric acid must contain 36·5 grams of the acid in a litre of fluid, and sodic hydrate 40 grams.

In the case of bivalent substances such as lead, calcium, oxalic acid, sulphurous acid, carbonates, etc., the equivalent is one-half

[1] *Lehrbuch der Chemisch-Analytischen Titrirmethode.*

of the atomic (or, in the case of salts, molecular) weight; thus a normal solution of oxalic acid would be made by dissolving 63 grams of the crystallised acid in distilled water, and diluting the liquid to the measure of 1 litre.

Further, in the case of trivalent substances such as phosphoric acid, a normal solution of sodic phosphate would be made by weighing $\frac{358}{3} = 119{\cdot}3$ grams of the salt, dissolving in distilled water, and diluting to the measure of 1 litre.

One important point, however, must not be lost sight of, namely, that in preparing solutions for volumetric analysis the value of a reagent as expressed by its equivalent hydrogen-weight must not always be regarded, but rather its particular reaction in any given analysis; for instance, with a solution of potassic permanganate $MnKO_4$, when used as an oxidising agent, it is the available oxygen which has to be taken into account, and hence in constructing a normal solution one-fifth of its molecular weight, $\frac{158}{5} = 31{\cdot}6$ grams, must be contained in the litre.

Illustrations may be given in order to show the method of calculating the results of this kind of analysis:—

1. Suppose, for instance, that it is desired to know the quantity of pure silver contained in a shilling. The coin is first dissolved in nitric acid, by which means a bluish solution, containing silver, copper, and probably other metals, is obtained. It is a known fact that chlorine combines with silver in the presence of other metals to form chloride of silver, which is insoluble in nitric acid. The proportions in which the combination takes place are 35·5 of chlorine to every 108 of silver; consequently, if a standard solution of pure chloride of sodium is prepared by dissolving in distilled water such a weight of the salt as will be equivalent to 35·5 grams of chlorine (= 58·5 grams NaCl) and diluting to the measure of 1 litre (1000 c.c.), such a solution will be a normal one, and every single gram measure of this solution will combine with 0·108 gram of pure silver to form chloride of silver, which is precipitated to the bottom of the vessel in which the mixture is made. In the process of adding the salt solution to the silver drop by drop (*titrating*), a point is at last reached when the precipitate ceases to form. Here the process is stopped.

On looking carefully at the graduated burette from which the standard solution has been used, the operator sees at once the number of c.c.'s which have been necessary to produce the complete decomposition. For example, suppose the *reading*, that is

to say, the number of c.c.'s used, equalled 33·7, all that is necessary to be done is to multiply 33·7 by the coefficient for each c.c., viz., 0·108, which shows the amount of pure silver present to be 3·63 grams.

Inversely, it is obvious that one may similarly test a substance for the quantity of chlorine by titrating a dissolved and measured quantity with a standard solution of silver.

2. Each c.c. of $\frac{N}{10}$ silver solution will contain $\frac{1}{10,000}$ of the atomic weight of silver = 0·0108 gram, and will exactly precipitate $\frac{1}{10,000}$ of the atomic weight of chlorine = 0·00355 gram from any solution of a chloride.

3. In the case of normal oxalic acid each c.c. will contain $\frac{1}{2000}$ of the molecular weight of the acid = 0·063 gram, and will neutralise $\frac{1}{2000}$ of the molecular weight of sodic carbonate = 0·053 gram, or will combine with $\frac{1}{2000}$ of the atomic weight of a dyad metal such as lead = 0·1035 gram, or will exactly saturate $\frac{1}{1000}$ of the molecular weight of sodic hydrate = 0·040 gram, and so on.

The great convenience of this equivalent system is, that the numbers used as coefficients for calculation in any analysis are familiar, and the solutions agree with each other, volume for volume.

We have hitherto looked only at one side of its advantages. For technical purposes, the plan allows the use of all solutions of systematic strength, and simply varies the amount of substance tested according to its equivalent weight.

Thus the normal solutions, say, are—

Sulphuric acid	49	grams	per litre
Nitric acid	63	,,	,,
Oxalic acid	63	,,	,,
Hydrochloric acid	36·5	,,	,,
Sodic hydrate	40	,,	,,
Sodic carbonate	53	,,	,,

100 c.c. of any one of the normal acids should exactly neutralise 100 c.c. of any of the normal alkalies, or the corresponding amount of pure substance which the 100 c.c. contain.

It is obvious that no experiment, however carefully performed, can be accurate unless the chemical reagents are pure and of the proper strength. It is also obvious that even if these conditions apply, proper quantities cannot be weighed without the balance is in perfect order, and that all burettes, pipettes, and

measuring flasks are perfectly graduated. It is therefore highly essential that these conditions should be carefully looked to, and particularly that burettes, pipettes, and measuring flasks be standardised, and re-marked with a file if practicable, or otherwise rejected and perfect ones obtained.

In the making up of all standard solutions a further essential is that the distilled water in use be perfectly pure and in most instances particularly free from ammonia.

We may now proceed to a consideration of the

Preparation of Standard and other Solutions.

To begin with, volumetric solutions may be divided into two groups :—

(*a*) *Permanent solutions*, *e.g.*, of sodium chloride, hydrochloric acid, and oxalic acid, which may be accurately prepared, and will keep well if properly stored. These may be regarded as true standards with which other solutions may be compared. If there be a doubt as to the exact strength of the standard, it is better, as a rule, to check it against a known weight of a suitable reagent rather than by some other standard solution.

(*b*) *Non-permanent solutions*, such as ferrous salts and dilute thiosulphate. With such it is a waste of time to aim at getting some definite strength. An approximation will suffice, the exact litre being determined each time of use by trial against some permanent standard, or a known weight of some reagent.

Normal Sulphuric Acid $\left(\frac{N}{\cdot}\right)$

= 49 grams H_2SO_4 per litre

1 c.c. =	0·049	gram	H_2SO_4
	0·048	,,	SO_4
	0·040	,,	SO_3
	0·090	,,	albumin
	0·017	,,	ammonia
	0·014	,,	nitrogen.

Run about 30 c.c. pure sulphuric acid, specific gravity 1·84, into a litre flask about a quarter full of distilled water; cool and make to mark at 60° F. (15·5° C.) with distilled water.

If the acid from which the solution has been made was of the specific gravity mentioned, the solution will be too strong, which is preferable, on account of the ease by which, in preference to a weak solution, it can be rendered accurate.

To test its strength several methods may be employed, the best being perhaps as follows:—

Weigh 2 grams of pure anhydrous sodic carbonate—recently heated and cooled under a desiccator — and place in a tared platinum dish. Dissolve in a small quantity of distilled water, covering the dish with a watch glass. Temporarily remove the watch glass and run in exactly 25 c.c. of the sulphuric acid to be standardised and immediately replace the watch glass. When the effervescence at first occurring has ceased, place the dish on the water bath and evaporate the contents to complete dryness, previously rinsing in any drops of liquid which may have collected on the inner surface of the watch glass. Now heat to 350° F. (176·6° C.) in an air bath, cool under desiccator, and weigh, repeating this until the weight is constant.

From this the exact strength of the made solution may easily be calculated.

The CO_2 in the sodic carbonate has been displaced by SO_3, and, as the atomic weight of the former is 44 as against 80 for the latter, it is evident that the increased weight of the residue over that of the sodic carbonate originally taken is proportional to the amount of sulphuric acid employed, provided an excess of the salt named be present and the quantity recommended ensures this being so.

Example:—

Platinum dish and Na_2CO_3	=	68·904 grams.
Platinum dish	=	66·808 ,,
		2·096

25 c.c. of the sulphuric acid of approximately normal strength were run in, and the whole evaporated to dryness, heated to 350° F. (176·6°C.) for some time, cooled under a desiccator and weighed. The dish and contents now weighed 69·382 grams, an increase of 0·478 grams. This increased weight is in proportion to the difference between the atomic weights of CO_2 and SO_3, that is, $44CO_2$ will be replaced by $80SO_3$, or the presence of 80 parts SO_3 will give an increased weight of 36. Therefore 36 : 80 : : 0·478 : 1·062 grams of SO_3 in 25 c.c. of dilute acid, or 4·248 grams per 100 c.c. in place of 4·00, the correct quantity. We therefore now dilute this as follows:—

4·00 : 100 : : 4·248 : 1·062.

That is, each 100 c.c. must be diluted to 106·2 to make it of correct strength.

The accuracy of this may be verified by making another experiment with the dilute liquid, either by evaporation with sodic carbonate as before, or as follows :—

Run 10 c.c. normal alkali into a small beaker or flask, add a few drops of litmus or methyl-orange, and allow the acid to flow from a 10 c.c. pipette, divided into $\frac{1}{10}$ c.c., until the point of neutrality is reached. If more than 10 c.c. are required, the acid is too weak; if less, too strong.

Suppose it required 8·9 c.c. to saturate the 10 c.c. of alkali, 890 c.c. will be required to make 1 litre of standard acid; remove, therefore, the excess from the litre flask and dilute to mark. Now test again with the pipette, and if the previous examination was correct, 10 c.c. of each solution should exactly neutralise each other.

As a further check upon the accurate strength of the solution, it is advisable to use larger quantities, say 50 or 100 c.c., for the final adjustment. The solution may also be controlled by precipitation with baric chloride, in which case 10 c.c. should produce as much baric sulphate as is equal to 0·49 gram of sulphuric acid, or 49 grams per litre.

Normal Nitric Acid $\left(\frac{N}{\cdot}\right)$

= 63 grams HNO_3 per litre
1 c.c. = 0·063 gram HNO_3
0·062 „ NO_3
0·054 „ N_2O_5.

Nitric acid used for making the normal solution should be colourless, free from chlorine and nitrous acid, and of a specific gravity of from 1·35 to 1·40. If the acid is coloured from the presence of nitrous acid, it should be mixed with an equal volume of distilled water and boiled until colourless. When cold it may be diluted and titrated against pure sodic carbonate, as described, with normal sulphuric acid.

Normal Oxalic Acid $\left(\frac{N}{\cdot}\right)$

= 63 grams $H_2C_2O_4$, $2OH_2$ per litre
1 c.c. = 0·063 gram $H_2C_2O_4$, $2OH_2$
0·045 „ $H_2C_2O_4$.

This solution possesses the advantage that it may be established directly, by weighing 63 grams of the pure crystallised acid,

dissolving the same in distilled water and making to the bulk of 1 litre at 60° F. (15·5° C.). The acid should be recrystallised, thoroughly air-dried, but not in the slightest degree effloresced. The solution keeps well, and will bear heating without volatilising the acid. The solution should be titrated against a normal solution of sodic carbonate.

NORMAL HYDROCHLORIC ACID $\left(\frac{N}{\cdot}\right)$

$$
\begin{aligned}
&= 36{\cdot}5 \text{ grams HCL per litre} \\
1 \text{ c.c.} &= 0{\cdot}0365 \text{ gram HCL} \\
& 0{\cdot}0355 \quad ,, \quad \text{Cl.}
\end{aligned}
$$

About 181 grams of pure hydrochlorlic acid (sp. gr. 1·10) made up to 1 litre at 60° F. (15·5° C.) with distilled water. The solution should be titrated against normal alkali.

NORMAL ALKALI.

Normal alkali may consist of either soda, potash, or (less recommendable) ammonia.

As pure sodic hydrate, manufactured from metallic sodium and free from carbonic acid, is readily obtainable, the standard solution is made from it as follows:—

NORMAL SODIC HYDRATE $\left(\frac{N}{\cdot}\right)$

$$
\begin{aligned}
&= 40 \text{ grams NaHO per litre} \\
1 \text{ c.c.} &= 0{\cdot}040 \text{ gram NaHO} \\
& 0{\cdot}031 \quad ,, \quad Na_2O \\
& 0{\cdot}06 \quad ,, \quad \text{acetic acid} \\
& 0{\cdot}09 \quad ,, \quad \text{lactic acid.}
\end{aligned}
$$

Dissolve about 42 grams sodic hydrate in about 800 c.c. distilled water, cool to 60° F. (15·5 C.). Titrate a portion of this solution with normal acid, calculate therefrom its exact strength and the dilution necessary to reduce it to 40 grams of actual NaHO per litre.

NORMAL SODIC CARBONATE $\left(\frac{N}{\cdot}\right)$

$$
\begin{aligned}
&= 53 \text{ grams } Na_2CO_3 \text{ per litre} \\
1 \text{ c.c.} &= 0{\cdot}053 \text{ gram } Na_2CO_3 \\
& 0{\cdot}030 \quad ,, \quad CO_3 \\
& 0{\cdot}022 \quad ,, \quad CO_2.
\end{aligned}
$$

Dissolve 53 grams of pure and dry sodic carbonate in distilled water and dilute to 1 litre at 60° F. (15·5° C.). The solution should be titrated against normal hydrochloric acid.

Decinormal Acid or Alkali $\left(\frac{N}{10}\right)$.

Run 100 c.c. normal acid or normal alkali into a litre flask about quarter full of distilled water, cool, and make to mark at 60° F. (15·5° C.) with distilled water.

Four-times Normal Acid or Alkali $\left(\frac{4}{N}\right)$.

Take 400 c.c. of strong hydrochloric acid and make up at 60° F. (15·5° C.) with distilled water to 1 litre. The solution may be titrated against normal alkali and appropriately diluted; each c.c. of the corrected standard should require 4 c.c. of normal alkali.

The corresponding four-times normal alkali is made by dissolving about 170 grams of sodic hydrate in distilled water, cooling, and making up to 1 litre. The solution may be titrated against four-times normal acid.

The acid solution is generally employed for inverting cane-sugar, and the corresponding alkali for neutralising the acidity after inversion. The solutions, however, need not be of exact strength.

Decinormal Potassic Permanganate $\left(\frac{N}{10}\right)$

$$
\begin{aligned}
&= 3{\cdot}156 \text{ grams } MnKO_4 \text{ per litre} \\
1 \text{ c.c.} &= 0{\cdot}0056\ Fe \\
& 0{\cdot}0072\ FeO \\
& 0{\cdot}0080\ Fe_2O_3 \\
& 0{\cdot}0017\ H_2S \\
& 0{\cdot}0056\ CaO.
\end{aligned}
$$

Weigh 3·156 grams pure crystal potassic permanganate and dissolve with a little distilled water (free from ammonia) in a litre flask and make to mark at 60° F. (15·5° C.). In standardising the solution it should react with its own volume of $\frac{N}{10}$ oxalic acid.

The solution should be kept in a well-stoppered bottle, and while it is quite free from sediment, it may be taken for granted

that its strength is unaltered. It will retain its strength for several months.

Decinormal Iodine $\left(\frac{N}{10}\right)$

$= 12 \cdot 7$ grams I per litre
1 c.c. $= 0 \cdot 0032$ gram SO_2.

Chemically pure iodine may be obtained by mixing commercial iodine with about one-fourth of its weight of potassic iodide and gently heating the mixture between two large watch glasses or porcelain capsules, the lower one being placed upon a heated iron plate, when the iodine sublimes in brilliant crystals which are absolutely pure.

The resublimed iodine of commerce is not always free from chlorine, and, unless purchased absolutely pure, it becomes necessary to prepare it by a second sublimation as described.

The watch glass or capsule containing the iodine is then placed under the desiccator so that the iodine may be cooled and freed from traces of moisture; then 12·7 grams are weighed and, together with about 18 grams of pure white potassic iodide, dissolved in about 250 c.c. distilled water.

The solution is then transferred to a litre flask, and made to mark at 60° F. (15·5° C.) with distilled water. The flask should not be heated in order to promote solution, and care should be taken that iodine vapours are not lost during the operation. The solution should be titrated against $\frac{N}{10}$ potassic permanganate:—

Take 10 c.c. of the permanganate, dilute with distilled water, acidify with sulphuric acid. Add potassic iodide, and titrate with $\frac{N}{10}$ sodic thiosulphate. Then titrate 10 c.c. of the $\frac{N}{10}$ iodine with the same thiosulphate solution. The volume consumed should be the same as in the previous titration.

Decinormal Sodic Thiosulphate $\left(\frac{N}{10}\right)$

$= 24 \cdot 8$ grams $Na_2S_2O_3$, $5OH_2$ per litre
1 c.c. $= 0 \cdot 01265$ gram I.

As it is not difficult either to manufacture or procure pure sodic thiosulphate, a portion is taken, powdered, and dried between blotting paper and 24·8 grams weighed. This quantity is then

dissolved in a little distilled water, transferred to a litre flask, and made to mark at 60° F. (15·5° C.) with distilled water.

The solution should be titrated with decinormal iodine solution, using a little gelatinised starch as an indicator. It should correspond, volume for volume, with $\frac{N}{10}$ iodine solution.

The solution is prone to decompose, depositing sulphur, especially if not kept in the dark. This tendency may be obviated, according to Mohr, by adding about 2 grams sesquicarbonate of ammonia.

O'Shaughnessy states that the addition of a little salicylic acid entirely prevents the solution decomposing.

Decinormal Sodic Chloride $\left(\frac{N}{10}\right)$

= 5·85 grams NaCl per litre
1 c.c. = 0·003537 gram Cl
0·005837 ,, NaCl.

5·85 grams of pure sodic chloride dissolved in distilled water and made up to 1 litre at 60° F. (15·5° C.).

Potassic Nitrate.

1·011 gram KNO_3 per litre
1 c.c. = 0·14 milligram N.

Dissolve 1·011 gram pure potassic nitrate and make to 1 litre at 60° F. (15·5° C.) with distilled water.

There is no simple volumetric method for checking this solution. If in doubt, make up a fresh one, as this will involve less time than the exact titration of the old solution.

Indigo Solution.

1 c.c. = 0·0001 gram N
0·000386 ,, N_2O_5.

Weigh 4 grams pure soluble indigo carmine in a small beaker; mix in a few drops of cold distilled water to form a paste, and then add 4 c.c. of strong sulphuric acid. Cover the beaker and allow to stand all night. Dilute with water, thoroughly mix, and make up at 60° F. (15·5° C.) to 1 litre.

The solution keeps for a lengthy period, but should be standardised against a standard solution of potassic nitrate (1·011

gram of the crystallised salt in 1 litre of distilled water). 5 c.c. of the nitrate solution is diluted to 50 c.c. with distilled water, sulphuric acid run in, and titration performed with the indigo as described under Water Analysis. 5 c.c. of the nitrate solution should require exactly 7 c.c. of the indigo.

Indigo solution to be used for tannin estimations need not be of exact strength.

Argentic Nitrate.

4·79 grams $AgNO_3$ per litre
1 c.c. = 0·001 gram Cl
0·00165 ,, NaCL.

Dissolve 4·79 grams pure recrystallised argentic nitrate in a little distilled water, transfer to a litre flask, and make to mark at 60° F. (15·5° C.) with distilled water.

The solution should be titrated against $\frac{N}{10}$ sodic chloride, using potassic chromate as indicator.

A decinormal solution may be prepared, if required, by dissolving 17 grams of the silver nitrate and diluting to 1 litre.

Each c.c. will then equal 0·010766 gram Ag.
0·016966 ,, $AgNO_3$.

Iron Solution.

1 c.c. = 0·1 milligram Fe.

Weigh 0·7 gram ferrous-ammonia sulphate, transfer to a boiling flask, dissolve in about 300 c.c. of distilled water, add about 5 drops nitric acid, and boil for about 10 minutes in order to oxidise the iron. Cool the solution, transfer to a litre flask, wash in the rinsings from the boiling flask, and make to mark at 60° F. (15·5° C.) with distilled water.

The formula of the ferrous-ammonia sulphate is $Fe(NH_4)_2(SO_4)_2 6OH_2 = 392$. Consequently, it contains exactly one-seventh of its weight of iron; 0·7 gram therefore represents 0·1 gram of iron, and this is a convenient quantity to weigh for the purpose of titrating permanganate solution hereafter referred to.

The solution is not permanent, and should be titrated before use. When freshly prepared, 100 c.c. of it should require 5·35 c.c. of $\frac{N}{30}$ permanganate.

Thirtieth Normal Potassic Permanganate $\left(\frac{N}{30}\right)$.

1 c.c. = ·001385 tannic acid.

Add 333·3 c.c. decinormal potassic permanganate to a litre flask and make to mark at 60° F. (15·5° C.) with distilled water free from ammonia.

The solution should be titrated against oxalic acid, 30 c.c. requiring 10 c.c. $\frac{N}{10}$ oxalic.

Sodic Thiosulphate for Forschammer's Oxygen Process.

Weigh 2 grams pure recrystallised sodic thiosulphate, transfer to a litre flask, dissolve in a little distilled water, and make to mark at 60° F. (15·5° C.) with distilled water.

The solution may be titrated against $\frac{N}{10}$ iodine.

Potassic Iodide for Forschammer's Oxygen Process.

Weigh 100 grams pure potassic iodide, dissolve in a little distilled water, transfer to a litre flask and make to mark at 60° F. (15·5° C.) with distilled water free from ammonia. The solution is not required to be very exact; it may be titrated, however (in the same way as a chloride), with silver nitrate, using potassic chromate as indicator: 5 c.c. of iodide should equal 30·1 c.c. $\frac{N}{10}$ silver solution.

Potassic Permanganate for Forschammer's Oxygen Process.

1 c.c. = ·0001 gram available oxygen.

Dissolve 0·395 gram pure dry potassic permanganate and make to a litre at 60° F. (15·5° C.) with distilled water free from ammonia.

The solution may be titrated against $\frac{N}{10}$ oxalic acid: 5 c.c. $\frac{N}{10}$ oxalic requires 40 c.c. permanganate.

So long as the solution is quite free from sediment, it may be taken for granted that its strength is unaltered.

Standard Ammonia.

Dissolve 3·15 grams ammonic chloride and make to 1 litre at 60° F. (15·5° C.) with distilled water free from ammonia.

The solution should be titrated against the silver nitrate solution previously described (4·79 grams $AgNO_3$ per litre), 10 c.c. requiring 20·9 c.c. of the silver solution.

Dilute Standard Ammonia.

1 c.c. = ·01 milligram NH_3.

Take 10 c.c. of the standard ammonia solution previously described and dilute to 1 litre with distilled water at 60° F. (15·5° C.).

The solution should be freshly prepared at least once a month.

Nessler's Solution.

Dissolve 62·5 grams potassic iodide in about 250 c.c. distilled water, set aside a few c.c., and add gradually to the larger part a cold, saturated solution of corrosive sublimate until the iodide of mercury precipitated ceases to be redissolved on stirring. When a permanent precipitate is obtained, restore the reserved potassic iodide so as to redissolve it, and continue adding corrosive sublimate gradually until a slight precipitate remains undissolved. (The small quantity of potassic iodide is set aside merely to enable the mixture to be made rapidly without danger of adding an excess of corrosive sublimate.)

Next dissolve 150 grams potassic hydrate in 150 c.c. distilled water, allow the solution to cool, add it gradually to the above solution, and make up with distilled water to 1 litre.

On standing, a brown precipitate is deposited, and the solution becomes clear and of a pale, greenish-yellow colour. It is ready for use as soon as it is perfectly clear, and should be decanted into a bottle as required.

Water Free from Ammonia.

When determining the amount of free or saline ammonia in a water, the sample is distilled which causes the free or saline ammonia, if present, to come off with the distillate. For this reason distilled water usually contains a considerable quantity of free ammonia and should therefore be tested before use. Unless free, it is useless to employ it for making up standard ammonia solutions or for Nesslerising purposes. If a good tap-water is at hand, showing no reaction of ammonia, it may be employed for Nesslerising, but failing the possession of such a water, it becomes

necessary to prepare distilled water free from ammonia; whilst owing to tap-water containing, as a rule, large quantities of saline bodies, it should not, even if free from ammonia, be employed for making up standard solutions.

It is an easy matter to expel the free ammonia from distilled water by vigorously boiling it in a capacious flask for about 30 minutes; the water being afterwards cooled and transferred to a bottle ready for use.

On the other hand, ordinary tap-water may be distilled rejecting the distillate so long as any colour is produced by Nessler's reagent; or the water in the retort may be acidulated with pure sulphuric acid, when the whole distillate will be free from ammonia and in all other respects pure.

Alkaline Potassic Permanganate.

Dissolve 200 grams potassic hydrate and 8 grams crystallised potassic permanganate with 800 c.c. distilled water in a large porcelain dish and evaporate over water-bath almost to dryness. This effectually expels all traces of ammonia. Cool, transfer to a litre flask, and make to mark at 60° F. (15·5° C.) with distilled water free from ammonia.

Ammonia Molybdate Solution.

Dissolve 4 grams molybdenum trioxide (MoO_3) in 94 c.c. ammonia (sp. gr. 0·880) and 150 c.c. distilled water; filter into 694 c.c. strong nitric acid (sp. gr. 1·2), dilute to a litre with a 12 per cent. solution of nitric acid; stand about 12 hours at 122° F. (50° C.) and then filter through three folded papers and bottle.

Magnesia Mixture.

Dissolve one part of crystallised magnesic sulphate and two parts ammonic chloride in eight parts of distilled water and four parts strong ammonia.

Allow the mixture to stand for several days and then filter and bottle.

Fehling's Solution.

Volumetric.

50 c.c. = ·24 gram dextrose.
·25 ,, invert.
·40 ,, maltose.

Gravimetric.

1 gram CuO = ·4535 dextrose.
·4715 invert.
·7435 maltose.

The solution consists of copper sulphate, potassic sodic tartrate (Rochelle salt), and sodic hydrate, and it was formerly customary to make and keep the solution in one bottle ready for use. It was found, however, that the mixed solution would not keep, but that under certain conditions, namely, provided the copper sulphate is made up as a separate solution and slightly acidified, it will keep sound for an almost indefinite period, whilst the alkaline solution kept separately will also keep for a lengthy period. The latter solution is not, however, so permanent as the copper sulphate, but as it need not be of accurate strength, it becomes an easy matter to prepare fresh solutions from time to time.

Two solutions are therefore now employed, viz., copper sulphate solution and alkaline tartrate, and are prepared as follows:—

Copper Sulphate Solution.—About 70 grams of crystallised copper sulphate are powdered and pressed between blotting paper; 69·28 grams are then carefully weighed and dissolved in about 400 c.c. distilled water, about 4 c.c. concentrated sulphuric acid then added, and the solution made to 1 litre at 60° F. (15·5 C.) with distilled water.

The solution should be standardised as hereafter described.

Alkaline Tartrate Solution.—346 grams of Rochelle salt and 130 grams of caustic soda are dissolved in about 800 c.c. distilled water, cooled, and made to 1 litre at 60° F. (15·5° C.) with distilled water. The solution should then be allowed to stand for 24 hours, and be afterwards filtered through a funnel containing a plug of glass wool.

Standardisation. Volumetric.—It is necessary to first prepare a standard solution of invert-sugar as follows:—

The purest variety of cane-sugar is that known as coffee-sugar, which generally contains from 99 to 99·8 per cent. of absolute sugar. The large crystals of this sugar should be selected, and after examining a solution in the polarimeter or determining the moisture percentage by drying a portion in the water oven, 2 grams of actual sugar should be weighed, that is, making allowance for the amount of moisture.

The 2 grams of actual sugar are now placed in a 200 c.c.

flask, 50 c.c. of distilled water and 5 c.c. of $\frac{4}{N}$ hydrochloric acid added, and the whole maintained at a temperature of 150° F. (65·5° C.) on the water bath for 20 minutes. By this time the sugar will have been inverted to dextrose or invert-sugar; the flask is then cooled and the solution neutralised by the addition of 5 c.c. $\frac{4}{N}$ alkali, the bulk being afterwards made up to 200 c.c. at 60° F. (15·5° C.) and a Fehling's test performed with some of the sugar solution in order to determine the quantity of Fehling's required. Having determined this, and knowing by calculation what quantity correct Fehling's should require, we may ascertain the dilution necessary to bring the copper sulphate solution to the proper strength.

Example.—The coffee-sugar taken was found by the polarimeter to contain 99·6 per cent. of real cane sugar; 2 grams of sugar were required, so 99·6 : 100 : : 2 : 2·008. 2·008 grams of the sugar were weighed and inverted with 5 c.c. $\frac{4}{N}$ acid, neutralised with 5 c.c. $\frac{4}{N}$ alkali and diluted to 200 c.c. Every 95 parts of cane-sugar on inversion become 100 parts of invert, hence 95 : 100 : : 2 : 2·10 grams invert-sugar in 200 c.c. and $2{\cdot}10 \div 2 = 1{\cdot}05$ in 100 c.c.

If 100 c.c. contain 1·05 gram of invert-sugar, how many c.c. will contain ·25 gram?—the quantity corresponding to 50 c.c. Fehling's.

1·05 : 100 : : ·25 : 23·8.

Therefore 23·8 c.c. of the sugar solution are required to reduce 50 c.c. Fehling's.

We now conduct a Fehling's test in order to ascertain if this is so or what the actual strength of the Fehling's is. To do this it should be remembered that the sugar solution experimented with should not contain more than 1 per cent. of sugar. In our example it will be noted that 2 grams of sugar diluted to 200 c.c. equals a 1 per cent. solution, so that the sugar solution is not above the maximum concentration for a Fehling's test. We therefore take 200 c.c. of distilled water in a boiling flask of about 800 c.c. capacity and boil. When well boiled, 50 c.c. Fehling's solution are added, that is, 25 c.c. of the standard copper solution and 25 c.c. of the alkaline tartrate.

The contents of the flask are again raised to the boiling-point, and 10 c.c. of the 1 per cent. sugar solution are run in from a

graduated burette. The whole is again boiled for about two minutes, and further quantities of the sugar solution are added at the rate of 5 c.c. at a time, the boiling being continued after each addition until a bright red colour, due to the formation of cuprous oxide (CuO), is obtained. The flask is now removed and set aside for the precipitate to settle. If the flask is now held to the daylight, the colour may readily be seen; should any blue tinge remain, more sugar solution, 1 c.c. at a time, is added, and the boiling recommenced and continued until the blue colour just disappears. To test whether the reduction of the copper is complete, a little of the solution is withdrawn by a glass rod and brought at once in contact with a drop of ferrous thiocyanate on a porcelain or opal glass slab.

It is always advisable to test before it is thought that the Fehling's is quite completed, as it is impossible to tell how much it is overdone, but absolutely easy to ascertain the incompletion. If it has been overdone, it is necessary to repeat the estimation over again with fresh Fehling's and further quantities of the sugar solution. It is almost impossible to hit the exact point on the first trial, but it affords a very good guide for a more exact titration the second time, and a second titration should always be made. When the titration is once commenced it should be carried on as quickly as possible, in order to prevent irregularities from long exposure of the hot solution to the atmosphere.

The reduction being complete, the number of c.c. of sugar solution used is read off from the burette. Let us assume we have used 31·4 c.c. sugar solution. We have seen from the calculation that 23·8 c.c. of the invert-sugar solution should reduce 50 c.c. Fehling's; but it is here found that 31·4 c.c. were required, so that the Fehling's solution is too strong.

What factor does 31·4 c.c. correspond to?

$$23{\cdot}8 : {\cdot}25 :: 31{\cdot}4 : {\cdot}320.$$

Therefore each 50 c.c. of Fehling's, or 25 c.c. of copper solution, equal ·320 invert-sugar instead of ·25.

How many c.c. of correct standard copper solution should represent ·320 grams?

If 25 c.c. equal ·25, how many c.c. does ·320 equal?

$${\cdot}25 : 25 :: {\cdot}320 : 32{\cdot}0 \text{ c.c.}$$

So we have merely to dilute 25 c.c. of our Fehling's solution

to 32 c.c. to bring it to proper strength, or add to each 25 c.c. that are left 7 c.c. of distilled water.

For particulars of gravimetric estimation, see p. 213, Malt Wort Analysis.

Soluble Starch (Dry).

Take about 1 lb. purified potato starch, place in a clean Winchester quart bottle, and add 1000 c.c. of a 7·5 per cent. solution of hydrochloric acid (75 c.c. hydrochloric acid, specific gravity 1037·0, and 925 c.c. distilled water). Set aside the mixture at the room temperature—60°–65° F. (15·5°–18·3° C.)—for seven days, stirring daily.[1]

At the end of this time wash thoroughly by decantation, at first with tap-water, and later on with pure distilled water, until the washings are free from chloride—neutral to freshly prepared litmus paper.

Now collect the starch on a filter paper, place in a Buchner's funnel, pump as dry as possible, and then spread it out on a new unglazed porous plate. The starch should now be dried at a gentle heat—110° F. (43·3° C.), as quickly as possible—and then triturated in a porcelain mortar and rubbed through a fine hair sieve.

Soluble Starch Solution for Lintner's Diastatic Process.

The previously prepared dry soluble starch is dissolved in boiling water at the rate of 2 grams per 100 c.c. of water. The solution is then cooled to 70° F. (21·1° C.) for use. It should be perfectly mobile (not gelatinous), indicating perfect conversion into soluble starch, and show only a negligible reducing action on Fehling's solution. It should be neutral to litmus solution, and the distilled water employed for making up the solution should be absolutely pure.

Potassic Permanganate for Hop Tannin Process.

Dissolve 1 gram pure dry potassic permanganate in a little distilled water and make to 1 litre with distilled water at a temperature of 60° F. (15·5° C.). The distilled water should be free from ammonia.

[1] According to Brown and Morris (*Jnl. Chem. Soc.*, 1889, 450), if a 12 per cent. solution of hydrochloric acid is employed, 24 hours' digestion suffices.

The solution may be titrated against decinormal oxalic acid: 10 c.c. $\frac{N}{10}$ oxalic should require 31·6 c.c.

Indigo Solution for Hop Tannin Process.

Dissolve 5 grams pure indigo carmine in 500 c.c. distilled water; add 50 c.c. concentrated sulphuric acid, cool, and make to 1 litre at 60° F. (15·5° C.) with distilled water. Each c.c. of this solution should correspond to an equal amount of the previously prepared potassic permanganate.

Gelatine Solution for Hop Tannin Process.

Weigh 25 grams Nelson's gelatine, place in a beaker, add 250 c.c. distilled water, and set aside for about 6 hours. Now heat on the water bath until the gelatine is dissolved; next saturate with fairly pure sodic chloride and make up to 1 litre at 60° F. (15·5° C.) with a saturated solution of sodic chloride. Shake the solution, allow to stand for a few days, warm, and then filter bright into a bottle ready for use.

¼–Litre Bottles (10 per cent. Solutions).

Plumbic acetate.
Baric chloride.
,, hydrate.
Ammonic chloride.
,, oxalate.
,, carbonate.
,, phosphate.
,, nitrate.
Sodic phosphate.
,, carbonate.
,, hydrate.
,, bisulphate.
Potassic hydrate.
,, ferrocyanide.
,, chromate (free from Cl).
,, iodide.
Potassic chloride.
Sulphuric acid, dilute (1–3).
Hydrochloric acid, dilute (1–3).
Acetic acid, dilute (1–1).
Ammonic hydrate, dilute (1–3).

TABLE FOR THE SYSTEMATIC ANALYSIS OF ALKALIES, ALKALINE EARTHS, AND ACIDS.

Substance.	Formula.	Atomic Weight.	Quantity to be weighed so that 1 c.c Normal Solution = 1 per cent. of Substance.	Normal Factor.[1]
			grams.	
Ammonia . . .	NH_3	17	1·7	0·017
Ammonic carbonate .	$(NH_4)_2CO_3$	96	4·8	0·048
Acetic acid . . .	$C_2O_2H_4$	60	6·0	0·060
Baric hydrate . .	BaH_2O_2	171	8·55	0·0855
,, ,, (crystals)	$BaO_2H_2(OH_2)_8$	315	15·75	0·1575
,, carbonate . .	$BaCO_3$	197	9·85	0·0985
Calcic oxide . .	CaO	56	2·8	0·028
,, hydrate . .	CaH_2O_2	74	3·7	0·037
,, carbonate . .	$CaCO_3$	100	5·0	0·050
Citric acid . . .	$C_6O_7H_8+OH_2$	210	7·0	0·070
Hydrochloric acid .	HCL	36·5	3·65	0·0365
Magnesia . . .	M_9O	40	2·00	0·022
Magnesic carbonate .	M_9CO_3	84	4·20	0·042
Nitric acid . . .	HNO_3	63	6·3	0·063
Oxalic acid . . .	$C_2O_4H_2$	126	6·3	0·063
Potash . . .	K_2O	94	4·7	0·047
Potassic hydrate . .	KHO	56	5·6	0·056
,, carbonate .	K_2CO_3	138	6·9	0·069
,, bicarbonate .	$KHCO_3$	100	10·0	0·100
Soda	Na_2O	62	3·1	0·031
Sodic hydrate . .	NaHO	40	4·0	0·040
,, carbonate . .	Na_2CO_3	106	5·3	0·053
,, bicarbonate .	$NaHCO_3$	84	8·4	0·084
Sulphuric acid . .	H_2SO_4	98	4·9	0·049
Strontia . . .	SrO	103·5	5·175	0·05175
Strontic carbonate .	$SrCO_3$	147·5	7·375	0·07375
Tartaric acid . .	$C_4O_6H_6$	150	7·5	0·075

[1] This is the coefficient by which the number of c.c. of normal solutions used in analysis is to be multiplied in order to obtain the amount of pure substance present in the material examined. See also *Multipliers required in Volumetric Analysis*, p. 394.

PART VI.

METHODS OF ANALYSIS.

Of the various chemical preparations offered for sale to brewers, it is necessary to know how much pure substance they contain. Let us first take, for instance, the various acids :—

Sulphuric Acid.

Take 10 c.c. of concentrated white sulphuric acid in a tared crucible and weigh. Suppose the weight is found to be 18·27 grams; then this, divided by 10, gives the specific gravity, viz. 1·827.

In consequence of the great concentration and high specific gravity of sulphuric acid, it is best to use only 1 or 2 c.c. for analysis; hence, after the specific gravity is ascertained, 2 c.c. may be titrated, taking care that a very fine and accurate pipette is used for the purpose; or, if this is not at hand, the acid may be weighed direct upon the balance.

Suppose we take 2 grams of the above acid in a porcelain dish, dilute with distilled water, add a few drops of methyl-orange or litmus solution, and titrate with normal alkali, 37·2 c.c. of the alkali being required; then

$$37{\cdot}2 \times 0{\cdot}049 \text{ (coefficient table)} = 1{\cdot}822, \text{ and}$$

$$\frac{1{\cdot}822 \times 100}{2} = 91 \text{ per cent. hydrated acid,}$$

which agrees with Otte's table (p. 401).

Hydrochloric Acid.

Take 5 c.c. of white hydrochloric acid in a tared crucible and weigh. Suppose the weight to be 5·6 grams; then this, divided by 5, gives the specific gravity as 1·12.

Dilute with distilled water and titrate with normal alkali, using a few drops of methyl-orange or litmus as indicator. Suppose 37·1 c.c. $\frac{N}{\cdot}$ alkali are required; this, multiplied by 0·0365 (coefficient table), gives 1·354 grams.

$$\frac{1{\cdot}354 \times 100}{5{\cdot}6} = 24{\cdot}1 \text{ per cent. hydrated acid.}$$

Ure's table (p. 402) gives 24·46 per cent. for the same specific gravity.

In order to ascertain the percentage of hydrochloric acid gas in any sample, it is only necessary to multiply the weight of gas found by normal alkali by 100, and divide by the weight of acid originally taken for analysis: the quotient will be the percentage. Or, simpler than this, if the $\frac{1}{10}$ equivalent in grams 3·646 grams be weighed, the number of c.c. will be the percentage.

Nitric Acid.

Take 5 c.c. of pure nitric acid and weigh. Suppose the weight to be 6·055 grams; this, divided by 5, gives the specific gravity as 1·211. Say the quantity of $\frac{N}{\cdot}$ alkali required to saturate the 5 c.c., using methyl-orange as indicator, is 28·3 c.c. This, multiplied by the coefficient factor 0·063, gives 1·782.

$$\frac{1{\cdot}782 \times 100}{6{\cdot}055} = 29{\cdot}4 \text{ per cent.}$$

Acetic Acid.

In consequence of the anomaly existing between the specific gravity of acetic acid and its strength, the hydrometer gives no uniformly reliable indication of the latter, and consequently the volumetric method is peculiarly suitable for ascertaining the value of acetic acid in all its forms. For most purposes normal caustic alkali may be used as the saturating agent; but a slight error occurs in this method, from the fact that neutral acetates have an alkaline reaction on litmus; the error, however, is very small, if care be taken to add the alkali till a distinct blue colour is reached. As acetic acid is volatile at high temperatures, normal carbonate of soda must not be used for titrating it, as it would necessitate heat to expel the carbonic acid.

Five c.c. of acetic acid weighs, say, 5·206 grams $\div 5 = 1{\cdot}041$ specific gravity. The quantity of normal alkali required to

saturate it is, say, 27·1 c.c.; then this multiplied by 0·06 (coefficient) = 1·626 grams.

$$\frac{1{\cdot}626 \times 100}{5{\cdot}206} = 31{\cdot}2 \text{ per cent.}$$

For the ordinary vinegar or turned beer there is no necessity to take the specific gravity into question: 5 or 10 c.c. may be taken as 5 or 10 grams. It is advisable to copiously dilute highly coloured liquids, in order that the change in the colour, produced by the litmus, may be distinguished; recourse must also be had to litmus paper, upon which little streaks should be made from time to time with the stirring rod.

Sulphurous Acid.

$$1 \text{ c.c. } \frac{N}{10} \text{ iodine} = 0{\cdot}0032 \text{ gram } SO_2.$$

$$1 \text{ c.c. } \frac{N}{10} \text{ thiosulphate} = 0{\cdot}01265 \text{ gram iodine.}$$

1. Take 20 c.c. of the acid in a 200-c.c. flask and make to mark at 60° F. (15·5° C.) with recently boiled and cooled distilled water. Take 10 c.c. of this in a porcelain dish, add a few drops of freshly prepared gelatinised starch solution,[1] and titrate with decinormal iodine until a blue colour makes its appearance and becomes permanent on stirring.

Suppose 23·3 c.c. $\frac{N}{10}$ iodine are required to produce the blue colour. Then 23·3 × ·0032 (factor) = 0·0745 × 100 = 7·45 per cent. sulphurous acid.

2. It is invariably found that the result is below the truth, for not only is oxidation proceeding during the time occupied in adding the iodine, but volatilisation of the free sulphurous acid also, to a slight extent, takes place. The source of error may be reduced by conducting a second titration, running into a porcelain dish containing distilled water and gelatinised starch solution the amount of iodine consumed in the first instance (23·3 c.c.), and then adding to this the 10 c.c. of dilute sulphurous acid. A further addition from the burette of as much as 1 c.c. iodine may have to be made in order to produce the blue colour.

The result would therefore be 24·3 c.c. instead of the previously mentioned 23·3 c.c.

[1] See footnote, p. 172.

The exactness of the method may, however, be still further ascertained by the following method:—

3. Take 10 c.c. of the prepared dilute acid solution in a porcelain dish, and add a few drops of gelatinised starch solution. In the first experiment 23·3 c.c. $\frac{N}{10}$ iodine were required, we therefore now add this quantity, together with an extra 5 c.c. $= 28·3$ c.c., and titrate with $\frac{N}{10}$ thiosulphate.

Suppose 7·1 c.c. $\frac{N}{10}$ thiosulphate are required to *change* the blue colour, then

$7·1 \times ·01265$ (factor) $= ·0898 \times 100 = 8·98$ per cent. sulphurous acid.

However, 5 c.c. extra iodine have been used, which are equal to 1·60 per cent. $(5 \times ·0032 = ·0160 \times 100)$, so that 1·60 from the above $8·98 = 7·38$ per cent.; and as the amount found in the first experiment with iodine was 7·45 per cent., the one experiment confirms the accuracy of the other, and the acid may be said to contain 7·45 per cent. of sulphurous acid.

Thiosulphate solutions are prone to change; it is advisable, therefore, to make a blank experiment with iodine; and if this be done, it is of no importance that the thiosulphate be strictly decinormal, since its exact value, as compared with iodine, is easily calculated, and the multiplier ·01265 merely altered accordingly.

The principle of this beautiful method of analysis, discovered by Dupasquier, consists in the fact that free iodine converts sulphurous into sulphuric acid by decomposing water, the oxygen of which goes to the sulphurous and produces sulphuric acid; the hydrogen, being taken by the iodine, forming hydriodic acid.

The reaction between iodine and very dilute sulphurous acid is represented by the formula

$$SO_2 + I_2 + 2OH_2 = 2HI + H_4SO_4.$$

Bunsen found that the solution should not contain more than 0·5 per cent. of sulphur dioxide, since, if above this strength, the action is reversed, the irregularity of decomposition varying with the quantity of water present, and the rapidity with which the iodine is added. This irregularity is, however, now obviated, as shown by Mohr, by the use of ammonic, potassic, or sodic bicarbonate, which have no effect upon iodide of starch. Either of these salts, added in moderate quantity to the liquid sulphurous acid, or, in case of sulphites, dissolving them in the alkaline

solution previous to titration with iodine, overcomes the difficulty hitherto found in concentrated solutions.

This method can be applied to the determination of a great variety of substances with extreme accuracy.

Bodies which take up oxygen, and decolorise the iodine solution, such as sulphurous acid, sulphites, sulphuretted hydrogen. alkaline thiosulphates, etc., are brought into dilute solution, gelatinised starch solution added, and the iodine delivered in with constant stirring until a point occurs at which a final drop of iodine colours the whole blue—a sign that the substance can take up no more iodine, and that the drop in excess has shown its characteristic effect upon the starch.

In diluting the liquid acid or in dissolving sulphites, etc., the distilled water employed for dilution should be well boiled and cooled, since, as shown by Giles and Shearer,[1] the air dissolved in the water used for dilution interferes with the accuracy of the results. They adopt, therefore, the method of placing the sulphite in a known excess of $\frac{N}{10}$ iodine, and determining such excess by means of $\frac{N}{10}$ sodic thiosulphate.

Commercial sulphurous acid purchased by brewers for cleansing purposes, fining manufacture, etc., should always be purchased at a definite specific gravity, a convenient gravity being about 10·33°.

Ammonia.

Take 10 c.c. ammonia and weigh. Suppose the weight to be 9·67 grams ; this, divided by 10, gives the specific gravity as 0·967.

The quantity of normal acid required to saturate the 10 c.c., using methyl-orange as indicator, is, say, 46 c.c. ; then this multiplied by 0·017 (coefficient) = 0·782

$$\frac{\cdot 782 \times 10}{9 \cdot 67} = 8 \text{ per cent. ammonia } (NH_3).$$

Carius's table, 0·967 specific gravity, gives 8 per cent. (p. 405).

Sodic Carbonate.

If it were absolutely pure, 5·3 grams of it should require exactly 100 c.c. $\frac{N}{\cdot}$ acid to effect neutralisation. If we therefore weigh that quantity, bring it into solution with distilled water, add a

[1] *Jnl. Soc. Chem. Ind.*, April 1884.

few drops of phenacetolin as indicator, and deliver into the mixture normal hydrochloric acid from a 100 c.c. burette, the number of c.c. required to saturate it will show the percentage of pure sodic carbonate in the sample.

Suppose 90 c.c. $\frac{N}{\cdot}$ acid are required. Then the sample contains 90 per cent. sodic carbonate.

As a further example—90 c.c. × ·053 (coefficient factor) = 4·77.

$$\frac{4{\cdot}77 \times 100}{5{\cdot}3} = 90 \text{ per cent.}$$

Suppose, on the other hand, it is required to know the equivalent percentage of

DRY CAUSTIC SODA,

free and combined, contained in the above sample of soda ash.

This may be ascertained by calculation thus:—

The sample contains 90 per cent. of sodic carbonate.

$$\frac{\text{Sodic oxide} = 62}{\text{Sodic carbonate} = 106} = 0{\cdot}585$$

$90 \times 0{\cdot}585 = 52{\cdot}65$ per cent. sodic oxide.

Again, instead of calculating the result, we may proceed as follows:—

Weigh 3·1 grams of the soda ash, bring it into solution with water, add a few drops of phenacetolin, and titrate with normal acid; the number of c.c. acid required to saturate it is the percentage of sodic oxide.

Example:—

Reading $\frac{N}{\cdot}$ acid = 52·6 c.c. = 52·6 per cent. sodic oxide, or 90 per cent. carbonate.[1]

ALKALINE SOLUTIONS.

From the nature of the substance, or its being in solution, the percentage method previously employed cannot here be conveniently followed. For example, suppose we have a solution which from the flame coloration is seen to be *potash*; a weighed or measured quantity of it is brought under the acid burette and saturated exactly by normal hydrochloric acid, using phenacetolin as indicator.

[1] 52·6 × ·031 (coefficient factor) = 1·630. 3·1 : 1·630 : : 100 : 52·6 per cent. sodic oxide or 90 per cent. sodic carbonate.

It is advisable, owing to the usual high strength of these solutions, to weigh or measure only about 5 c.c. and dilute largely with distilled water so that the reaction may be clearly observed.

Supposing, of 5 c.c. taken, the amount of normal hydrochloric acid employed to saturate it is 37 c.c. The molecular weight of potassic hydrate being 56, 100 c.c. $\frac{N}{\cdot}$ hydrochloric acid will saturate 5·6 grams, therefore $\frac{5{\cdot}6 \times 37}{100} = 2{\cdot}072$; and as 5 grams of the solution were taken, $2{\cdot}072 \times 20 = 41{\cdot}44$ per cent. KHO.

On the other hand, suppose the specific gravity of the solution is found to be 1·380; this, by Tünnermann and Richter's table (p. 404), is equal to 41·37 per cent., against our 41·44 per cent. found by titration.

With *soda* solutions we preceed in precisely the same manner. For example, we have a solution which by the colour flame is seen to be soda. Of 5 c.c. taken, the amount of $\frac{N}{\cdot}$ hydrochloric acid employed to saturate it is, say, 23 c.c.

The molecular weight of sodic oxide being 62, 100 c.c. of $\frac{N}{\cdot}$ hydrochloric acid will saturate 6·2 grams, therefore $\frac{6{\cdot}2 \times 23{\cdot}0}{100} = 1{\cdot}426$; and as 5 c.c. were taken, $1{\cdot}426 \times 20 = 28{\cdot}52$ per cent. Na_2O.

The specific gravity is found, say, to be 1·40; according to Tünnermann's table (p. 404) = 28·40 per cent. against our 28·52 per cent. by titration.

Water Analysis.

The following are the determinations to be made:—

1. Total solids.
2. Saline residue.
3. Organic and volatile matter.
4. Lime.
5. Magnesia.
6. Sulphuric acid.
7. Chlorine and chloride of sodium.
8. Iron.
9. Alkalinity before and after boiling.
10. Soda and potash.

Nitrogen as Nitrates and Nitrites :—

11. Nitric acid.
12. Nitrous acid.

Organic Matter :—

13. "Free or saline" ammonia.
14. "Albuminoid" ammonia.
15. Oxygen required to oxidise organic matter.

The following further tests are sometimes necessary :—

16. Silica.
17. Lead.
18. Copper.
19. Bacteriological examination (see p. 369).

WATER ANALYSIS.

Collection of Samples.

Every precaution should be taken to ensure the collection of a truly representative sample. Stoneware bottles should be avoided, as they are apt to affect the hardness of the water, and are more difficult to clean than glass. Stoppered glass bottles should be used where convenient; those known as "Winchester Quarts," which hold about two-and-a-half litres each, are very handy and easy to procure. One of these will hold sufficient for the general analysis of a water, whilst two are ample for all purposes, including a bacteriological examination, should such be required.

If corks are used, they should be *new*, and well washed with the water at the time of collection.

In collecting from a well, river, or tank, plunge the bottle itself, if possible, below the surface; but if an intermediate vessel must be used, see that it is thoroughly clean and well rinsed with the water. Avoid the surface water and also any deposit at the bottom. If the sample is taken from a pump or tap, take care to let the water which has been standing in the pump or pipe run off before collecting, then allow the stream to flow directly into the bottle.

If it is to represent a town water supply, take it from the service pipe communicating directly with the street main, and not from a cistern.

In every case, first fill the bottle completely with the water, empty it again, rinse once or twice carefully with the water, and then fill it nearly to the stopper and tie down tightly.

At the time of collection note the source of the sample, whether from a deep or shallow well, a river, or spring. If it is from a

well, ascertain the nature of the soil, subsoil, and water-bearing stratum, the depth and diameter of the well, its distance from neighbouring cesspools, drains, or other sources of pollution; whether it passes through an impervious stratum before entering the water-bearing stratum, and if so, whether the sides of the well above this are, or are not, water-tight.

If the sample is from a river, ascertain the distance from the source to the point of collection; whether any pollution takes place above that point, and the geological nature of the district through which it flows.

If from a spring, take note of the stratum from which it issues.

In order to ensure uniformity, the bottle should invariably be well shaken before taking out a portion of the sample for the purpose of analysis.

Colour.

A very tolerable opinion may be formed as to whether a sample of water contains unchanged organic matter by comparing its colour with that of distilled water; to this end Lovibond's tintometer may be used, or, failing this, two white glass cylinders about 12 or 18 inches high should be placed upon white paper or a white porcelain slab, one filled with distilled water and the other with the sample to be tested, side by side: any yellow or brown colour in the sample indicates the presence of organic matter, but it may not necessarily be owing to very objectionable impurity, since purely vegetable matter such as peat will often produce it.

Smell.

The smell can be observed by shaking up some of the water in a large, wide-mouthed flask or bottle, and applying the nose to the bottle immediately afterwards. If the water be warmed to a slight extent, any objectionable smell is more readily detected.

Filtration.

Before commencing the quantitative analysis, it is necessary to decide whether the water shall be filtered or not. This must depend on the purpose for which the examination is undertaken. As a general rule, if the suspended matter is to be determined, the water should be filtered before the estimation of ammonia and total solid residue; if otherwise, it should merely be shaken up.

If the suspended matter is not determined, the appearance of the water, whether clear or turbid, should be noted.

Water derived from a newly sunk well, or which has been rendered turbid by the introduction of innocuous mineral matter from some temporary and exceptional cause, should be filtered; but the suspended matter, in most cases, particularly with brewery well waters, need not be determined.

Suspended Matter.

From a sanitary point of view suspended matter is of minor interest, because it may be in most cases readily and completely removed by filtration. Mineral suspended matter is, however, of considerable mechanical importance as regards the formation of impediments in the river-bed by its gradual deposition, and as regards the choking of filters and the binding effect it has with the saline bodies causing incrustation in boilers.

1. **Total Solids.**—Evaporate over the water bath half a litre (500 c.c.) or a less quantity of the water, measured at 60° F. (15·5° C.), in a platinum dish which has been heated to redness, cooled under the desiccator, and carefully weighed.

As soon as the evaporation is complete, the dish with the residue is removed from the water bath, its outer side wiped dry with a cloth, and then dried in the oven for about three hours. It is then placed under the desiccator, allowed to cool, weighed as rapidly as possible, returned to the oven, dried, cooled, and again weighed at intervals of an hour until the weight is constant.

Example:—

Weight of platinum dish and solids .	70·414 grams.
Weight of platinum dish . . .	60·420 ,,
	9·994

A gallon of water weighs 10 lbs. or 70,000 grains; when taking 500 c.c. multiply by ·2 to bring to 100 c.c., and by ·7 to obtain the number of milligrams in 70 c.c., which for the purpose of calculation is reckoned as a miniature gallon. Milligrams per 70 c.c. equal grains per gallon; hence $9·994 \times ·2 = 1·9988 \times ·7 = 1·39916 = 13·9916$ grains solid matter in 70 c.c., or **139·92 grains per gallon total solid matter.**

2. **Saline Residue.**—Having obtained the weight of the total solid matter, the platinum dish containing it is placed over a gas flame and gently ignited. If, on being heated, the solid residue darkens, it indicates a large amount of organic matter; and if it becomes black, the water is highly contaminated. The ignition is carried on for about half a minute until the residue is quite

white or brown, the latter showing the presence of iron. The dish is then cooled, the residue recarbonated, by moistening it with a drop or two of ammonic carbonate, and again gently heated over the gas flame, care being taken that the carbonate is not driven off during the heating. The dish is once more cooled under the desiccator and again weighed at intervals until constant.

Example:—

Weight of platinum dish and saline residue	69·380 grams.
Weight of platinum dish . . .	60·420 ,,
	8·960

8·960 × ·2 = 1·7920 × ·7 = 1·2544, or **125·40 grains per gallon saline residue.**

3. **Organic and Volatile Matter.**—The difference between the total solid matter and the saline residue, thus:—

Total solid matter	=	139·920 grains per gallon.
Saline residue	=	125·400 ,, ,,
		14·520 grains per gallon organic and volatile matter.

4. **Lime.**—Take 500 c.c. of the water at 60° F. (15·5° C.) in a large beaker, add 5 or 6 drops of hydrochloric acid, and boil down to about half bulk. Now add about 20 c.c. of 10 per cent. solution ammonic oxalate, about 10 c.c., 10 per cent. ammonic chloride, and about the same quantity of 10 per cent. ammonic hydrate, which cause the lime to separate in the form of calcic oxalate.[1] The liquid should now be set aside and allowed to

[1] The object of the addition of ammonic hydrate is as follows:—

Lime may be precipitated in neutral or fairly acid solution by ammonic oxalate, but the precipitation is in such case incomplete. In ammoniacal solutions, however, the precipitation is perfect.

The addition of ammonic chloride is unnecessary for the separation of calcic salts, but it is added to prevent the simultaneous precipitation of magnesia, which also forms an insoluble oxalate, but not in the presence of ammonic chloride. A quantity of ammonic oxalate should therefore be added sufficient not only to convert the calcic, but also the magnesic, salts into oxalates. Fresenius shows that in the presence of large quantities of magnesia this process is not rigidly accurate, but that a small quantity of this body precipitates along with the lime; and further, that unless a large excess of ammonic oxalate is present, the lime is not completely precipitated. He considers a re-solution and precipitation of the calcic oxalate a necessity, but waters as a rule do not contain sufficient magnesia to render such operation necessary.

stand for not less than 3 hours, by which time the precipitation will be complete and the supernatant liquid bright.

Nearly the whole of the liquid is now carefully filtered into a clean dry beaker, through a filter paper previously moistened with water, the precipitated insoluble calcic oxalate, together with the small quantity of liquid left in the beaker, being then stirred up by means of a glass rod to the end of which is attached a small piece of rubber tube; the liquid is then passed on to the filter, the beaker being rinsed with distilled water, any particles of calcic oxalate being removed by the glass rod, and the repeated washings in this manner transferred to the filter until every particle of the oxalate has been transferred to the filter. The filter paper is now washed by sparging successive portions of boiling distilled water from the wash bottle, and these washings also collected in the beaker containing the filtrate. If the filtrate is cloudy, it must be again returned to the filter paper and filtered until perfectly brilliant; there is, however, seldom any difficulty in this respect with a filtration of a lime precipitate.

The filtrate from the lime is required for the subsequent estimation of magnesia, and is therefore set aside until required.

The filter paper containing the calcic oxalate is now placed in the water oven and dried, after which it is carefully folded, put in a tared crucible, and placed on a tripod over a Bunsen flame and the oxalate burned to a white or grey ash, the oxalate by this ignition being reduced to carbonate. Very slight heat is sufficient to decompose calcic oxalate into calcic carbonate, but small quantities of carbon-dioxide are disengaged from the carbonate so that it is advisable to "recarbonate." This is effected by moistening the ash, after cooling, with two or three drops of ammonic carbonate solution, then carefully drying on the water bath, and finally gently heating for a moment or two over a small flame. Any carbon-dioxide which may have been volatilised in the first heating is thus replaced at the expense of the ammonic carbonate, while the excess of the latter is volatilised on drying in the water bath and subsequent exposure to gentle heat over the flame.

The crucible is now placed under the desiccator to cool and is then weighed, the ash being again recarbonated, dried, cooled, and weighed until the weight is constant. The amount of calcic carbonate ($CaCO_3$) so found is now calculated to that of calcic oxide (CaO).

The atomic weight of $CaCO_3$ is 100, and that of CaO 56,

therefore every 100 parts of $CaCO_3$ contain 56 parts of CaO; the quantity of calcic oxide is therefore calculated as follows:—

$$\frac{56 \times CaCO_3 \text{ found}}{100} = CaO.$$

Or the same thing is arrived at by multiplying the $CaCO_3$ found by ·56.

Example:—

Weight of crucible and precipitate .		8·027 grams.
Weight of crucible		7·564 ,,
	$CaCO_3$	·463

Then $\cdot463 \times \cdot2 = \cdot0926$ grams $CaCO_3$ in 100 c.c.
$\cdot0926 \times \cdot7 = \cdot06482$,, $CaCO_3$ in 70 c.c.; or
64·82 grains per gallon $CaCO_3$, and
$64\cdot82 \times \cdot56 =$ **36·33 grains per gallon of lime (CaO).**

5. **Magnesia.**—To the filtrate from the lime add about 10 c.c. of 10 per cent. solution ammonic or sodic phosphate and about 10 c.c. of strong ammonic hydrate, well stir, and allow to stand for 6 hours, or preferably over-night. Filter, wash the precipitate with dilute ammonia, dry, place in a tared crucible, and strongly ignite to a grey ash; cool under desiccator and weigh as pyrophosphate of magnesia $2(MgO)P_2O_5$.

The atomic weight of $2(MgO)P_2O_5$ is 222, and that of 2MgO, 80; every 222 parts of the pyrophosphate therefore correspond to 80 of magnesia, or

$$\frac{80}{222} = \cdot36.$$

The factor ·36 may be employed to convert the one into the other.

Example:—

Crucible and precipitated pyrophosphate	7·765 grams.
Crucible	7·564 ,,
	·201

Then $\cdot201 \times \cdot2 = \cdot0402 \times \cdot7 = \cdot02814$ per 70 c.c.
So that $28\cdot14 \times \cdot36 =$ **10·15 grains per gallon magnesia (MgO).**

6. **Sulphuric Acid.**—Take 500 c.c. of the water at 60° F. (15·5° C.) in a large beaker, add three or four drops of hydrochloric acid, boil to half bulk, and add about 15 c.c. of 10 per

cent. solution baric chloride. Boil for a further two or three minutes and then set aside in a warm place so that the baric sulphate may settle. When the supernatant liquid is clear, filter [1] and wash the precipitate thoroughly with boiling distilled water. Now place the filter containing the precipitate in the water oven to dry, then ignite to a white ash in a tared crucible, cool under the desiccator and weigh, repeating ignition, cooling, and weighing until the weight is constant.

Example:—

Weight of crucible and baric sulphate .	8·739 grams
Weight of crucible	7·650 ,,
	1·089

Therefore $1·089 \times ·2 = 2·178 \times ·7 = 1·5246$ per 70 c.c., or 15·246 grains per gallon $BaSO_4$.

It now remains to ascertain the quantity of sulphuric anhydride corresponding to the weight of baric sulphate obtained. The atomic weight of $BaSO_4$ is 233, and that of sulphuric anhydride (SO_3), 80; 233 parts of baric sulphate therefore correspond to 80 parts of sulphuric anhydride, hence $\frac{80}{233} = ·343$; so that we have merely to multiply by the factor ·343. Thus 15·246 grains per gallon $BaSO_4 \times ·343 =$ **52·29 grains per gallon sulphuric anhydride (SO_3).**

7. **Chlorine and Chloride of Sodium.**—To 250 c.c. of the water at 60° F. (15·5° C.) in a large porcelain dish, add two or three drops 10 per cent. potassic chromate (free from chlorine), so as to give it a faint tinge of yellow, and add gradually from a burette standard argentic nitrate solution (p. 137) until the red argentic chromate which forms after each addition of the nitrate ceases to disappear on stirring.

The chromate is simply an indicator, the affinity of silver for chlorine being greater than that for chromic acid; hence no chromate of silver is found until the chlorides in the water are used up.

Where extreme accuracy is desired, after completing a determination, destroy the slight red tint by an excess of a soluble chloride, and repeat the estimation on a fresh quantity of the

[1] It is sometimes difficult to obtain a clear filtrate owing to the sulphate forming a very finely divided precipitate; this may be avoided by the addition at the time of adding the baric chloride, of about ·005 gram of potato starch which has a conglomerating action upon the precipitate and retains it on the filter paper. Repeated filtration, however, is effectual.

water in a similar porcelain dish placed by the side of the former. By comparing the contents of the dishes, the first tinge of red in the second dish may be detected with great accuracy.

It is absolutely necessary that the liquor examined should not be acid, unless from carbonic acid, nor more than slightly alkaline. It must also be colourless or nearly so. These conditions are generally found in waters; but, if not, they may be brought about in most cases by rendering the liquid just alkaline with lime water (free from chlorine), passing carbonic anhydride to saturation, boiling, and filtering. The calcic carbonate has a powerful clarifying action, and the excess of alkali is exactly neutralised by the carbonic anhydride. If this is not successful, the water must be rendered alkaline, evaporated to dryness, and the residue *gently* heated to destroy organic matter. The chlorine may then be extracted with distilled water and estimated in the ordinary way.

Example.—8·5 c.c. argentic nitrate solution were required to produce the reddish tint in 250 c.c. of the water. Then $8{\cdot}5 \times {\cdot}4 = 3{\cdot}40 \times {\cdot}7 =$ **2·38 milligrams chlorine per 70 c.c. or grains per gallon.**

In order to convert chlorine to sodic chloride :—

The atomic weight of chlorine (Cl) is 35·5, and that of sodic chloride (NaCl) 58·5. Every 35·5 parts of chlorine therefore correspond to 58·5 parts of sodic chloride, hence $\frac{58{\cdot}5}{35{\cdot}5} = 1{\cdot}645$; so that we multiply by the factor 1·645, or, to simplify matters, by 1·65. Thus 2·38 grains per gallon chlorine $\times$ 1·65 = **3·92 grains per gallon sodic chloride.**

8. **Iron.**—Take 70 c.c. of the water at 60° F. (15·5° C.) in a small boiling flask, add 1 c.c. concentrated nitric acid (the presence of free acid is always necessary in this process in order to convert iron existing in the water in a ferric state to that of the ferrous state), and boil for 5 minutes. Cool to 60° F. (15·5° C.), transfer to a Nessler tube, and make up to 70 c.c. with distilled water. Add a drop or two of 10 per cent. solution potassic ferrocyanide, when, if iron is present, a blue colour will appear which is then imitated by a standard solution of iron (p. 137), added to a similar bulk of distilled water in a Nessler tube until the colour matches that of the sample, when both tubes are looked through while standing on a white surface.

The exact strength of the iron solution being known (1 c.c. = 0·1 milligram Fe), it is easy to arrive at the quantity of pure iron

present in the water, and to convert it into its state of combination by calculation.

Example.—In this case 1·4 c.c. of the standard iron solution were required to imitate the blue colour, each c.c. being equivalent to 0·1 milligram of iron.

$$1{\cdot}4 \times {\cdot}1 = {\cdot}14 \text{ milligram of iron in 70 c.c.}$$
$$\text{or } {\cdot}14 \text{ grain per gallon.}$$

It is usual, however, to express the iron as ferric oxide (Fe_2O_3), so that as 160 parts Fe_2O_3 equal 112 parts of iron, $\frac{112}{160} = 0{\cdot}7$. We have merely to multiply 0·14 by 0·7 to obtain the amount of ferric oxide, which, therefore, equals **0·098 grain per gallon Fe_2O_3.**

This method, which approaches in delicacy the Nessler test for ammonia, is applicable for very minute quantities of iron; in fact, 1 part of iron in 13,000,000 parts of water can thus be detected.

9. **Alkalinity before and after Boiling.**—This estimation is merely carried out as a check upon the accuracy of other analytical results, and is of great importance, particularly with waters containing carbonate of soda or potash.

Thus, after combining the acids and bases we have, say, a certain quantity of calcic carbonate, and a certain quantity of magnesic carbonate. By now finding the equivalent of the magnesic carbonate and expressing it as calcic carbonate, this, added to the actual amount of calcic carbonate originally found, shows the total alkalinity of the water; which, if the same as the alkalinity estimated by this test, shows that the analysis in this respect is correct. If, however, there is a deficiency, then the analysis is incorrect; whilst if there is an excess, it shows that such excess is due to carbonate of soda or potash.

The estimation is made as follows:—

(1) Measure 350 c.c. of the water at 60° F. (15·5° C.), run into a large beaker and titrate with $\frac{N}{10}$ acid, using two or three drops of methyl-orange as an indicator. Say, for instance, that 32·2 c.c. $\frac{N}{10}$ acid were required to produce the faint pink coloration.

(2) A further 350 c.c. of the water at 60° F. (15·5° C.) are now measured out into a large beaker and boiled for three-quarters of an hour. The liquid is then filtered through a paper previously moistened with boiling distilled water, the beaker rinsed with

distilled water, and the washings transferred to the filter. The filtrate, after cooling, is now titrated with $\frac{N}{10}$ acid, using methyl-orange as an indicator. Say, for example, that 1·6 c.c. $\frac{N}{10}$ acid were required to produce the faint pink coloration.

Now the difference between these two titrations is due to the precipitation of carbonate of lime during boiling, with perhaps a trace of carbonate of magnesia. Lime is not, however, absolutely insoluble in boiling water, but remains in solution to the extent of 1·26 grain per gallon, and this figure may therefore be taken into account when calculating the result. If 350 c.c. of the water are employed, each c.c. of $\frac{N}{10}$ acid corresponds to one grain of carbonate of lime per gallon.

Example:—

350 c.c. unboiled water required	.	32·2 c.c. $\frac{N}{10}$ acid.
350 c.c. boiled and filtered water	.	1·6 „ „
Alkalinity removed by boiling	.	30·6

grains per gallon $CaCO_3$. To this figure is added 1·26 (solubility of $CaCO_3$ in boiled water) = **31·8 grains per gallon $CaCO_3$.**

10. **Soda and Potash.**—Take 500 c.c. of the water at 60° F. (15·5° C.) in a beaker and boil down to about 50 c.c. Add baric hydrate until the liquid is alkaline (about 10 c.c.), and set aside in a warm place (on the water bath) for one hour. Filter and wash the precipitate well with hot distilled water. To the filtrate add about 5 c.c. of 10 per cent. ammonic chloride and ammonic carbonate until no more barium is precipitated (probably about 10 c.c.).

The barium precipitates the bases except the alkalies—soda and potash; and the ammonic chloride and carbonate precipitate the excess of barium, leaving the alkalies in solution as chlorides.

Now allow to stand for 2 hours, then filter and wash with hot distilled water, after which boil the filtrate down to about 25 c.c.; transfer to a clean platinum dish and evaporate to dryness. When dry, gently ignite until ammoniacal fumes cease to be given off, but taking care not to heat too strongly for fear of volatilising small quantities of chlorides. Cool under the desiccator and weigh; repeat ignition, cool, and again weigh. Now extract the residue with warm distilled water and filter. Return the filter paper to the platinum dish, ignite, cool, and weigh.

The difference between the former weight (which is the tare of the dish and traces of insoluble substances) and this one gives sodium and potassium as chlorides. It now remains to separate these alkalies. This is performed by adding 5 c.c. of platinic bichloride to the filtrate, which is then evaporated to dryness, dried at 212° F. (100° C.), cooled, and weighed until constant. The gain in weight is due to potash existing as potassic platinic chloride $(KCl_2)PtCl_4$. The potassic chloride being thus found, the quantity of sodic chloride is then calculated.

Example:—

Weight of platinum dish + solids before extraction	62·570 grams.
Weight of platinum dish + solids after extraction	62·462 ,,
	·108

·108 × ·2 = ·0216 × ·7 = 15·120 grains per gallon total chlorides.

After treatment with platinic chloride:—

Weight of platinum dish + solids .	60·450 grams.
Weight of platinum dish . .	60·418 ,,
	·032

·032 × ·2 = ·064 × ·7 = ·0448; therefore potassic platinic chloride = 4·48 grains per gallon and 4·48 × ·307[1] = 1·375 grain per gallon potassic chloride.

Total alkalies . .	15·120 grains per gallon.
Potassic chloride . .	1·375 ,, ,,
Sodic chloride . .	13·745

[2] NaCl 13·745 × ·53 = 7·25 grains per gallon Na_2O.
KCL 1·375 × ·63 = 0·86 ,, ,, K_2O.

It will be seen that the estimation of soda and potash is rather tedious, and by no means easy. Frequently, owing to incomplete separation of other bodies, the alkalies come out too high, and this error is attributed to the soda, owing to the potash being subsequently determined.

[1] $(KCl_2)PtCl_4$ = 484·5 gives 2KCl = 148·7, therefore 1 = ·307.

[2] 2NaCl = 116·7 gives Na_2O = 62·0, therefore 1 = ·53.
2KCl = 148·7 gives K_2O = 94·0, therefore 1 = ·63.

Nitrogen as Nitrates and Nitrites.

By far the best test for estimating nitrogen as nitrates and nitrites in water is that known as the mercury method, but it has the great disadvantages of requiring costly gas-analysis apparatus, of being complicated, and, in inexperienced hands, yields poor results. The indigo method about to be described is the one now generally resorted to, and when carefully performed gives excellent results. In fact the following will show the difference in the results obtained by the two processes:—

	Parts per 100,000.	
	With Mercury.	With Indigo.
1	0·973	0·912
2	4·235	4·530
3	1·825	1·706
4	0·729	0·676
5	2·749	2·912
6	1·696	1·294
7	2·144	2·265
8	0·354	0·338
9	2·860	2·824
10	0·222	0·221

The indigo process was originally devised by Marx[1] and improved by Warrington.[2]

The principle of the method is that of liberating free nitric and nitrous acids from their combinations by the aid of strong sulphuric acid, and measuring the quantity so liberated by the decoloration of a solution of indigo.

The accuracy of the method is disturbed in the presence of much chlorides, so that for such cases an alternative method, Gladstone and Tribe's, is also given.

11. **Estimation of Nitric Acid: Marx Indigo Process.**—Take 50 c.c. of the water at 60° F. (15·5° C.) in an 8 ounce flask and add an equal bulk of pure strong sulphuric acid, allowing the acid to run down the side of the flask and avoiding mixing the solutions as far as possible. Now agitate the contents of the flask and rapidly add, from a burette, a standard solution of indigo (p. 136), until a permanent faint bluish-green tint is perceptible. Read off the number of c.c. of indigo consumed, and as each c.c. corresponds to 0·1 milligram of nitrogen, the

[1] *Zeitschr. f. ang. Chem.*, vol. viii. 412.
[2] *Jnl. Chem. Soc. Trans.*, xxxv. 578.

amount of nitrogen in the 50 c.c. of the water is found by multiplying by ·01.

To find from this the corresponding amount of nitric acid is a simple matter. Two atoms of nitrogen (N_2) are contained in the molecule of nitric anhydride (N_2O_5); the calculation therefore is:—

$$N_2 = 28 \quad N_2O_5 = 108$$
$$\frac{108 \times \text{N found}}{28} = N_2O_5.$$

Or, by dividing the atomic weight of N_2 into that of N_2O_5, the factor 3·86 is obtained, which gives the amount of nitric acid (anhydrous) from the nitrogen found; and the result is then calculated to grains per gallon.

Example.—50 c.c. of water took 2·3 c.c. indigo solution $2{\cdot}3 \times 2 = 4{\cdot}6 \times {\cdot}7 = 3{\cdot}22$ per 70 c.c. water; $3{\cdot}22 \times {\cdot}1 = {\cdot}322$ gram N per gallon; $\cdot 322 \times 3{\cdot}86 =$ **1·25 grain per gallon nitric anhydride.**

Estimation of Nitric Acid: Gladstone and Tribe's Method.—This method was introduced by Gladstone and Tribe[1] twenty-eight years ago, and its application to water analysis was fully dealt with in 1881 by W. Williams.[2] It is accomplished as follows:—

Take 250 c.c. of the water at 60° F. (15·5° C.) in a porcelain dish, add two or three drops of caustic potash solution which must be free from ammonia, evaporate over the water bath to about 50 c.c., and then transfer to a small flask, the cork of which is provided with a thistle-headed funnel and stop-cock. Wash the porcelain dish with distilled water, and add the rinsings to the flask so as to make up the bulk to about 20 c.c. Now add a few pieces of copper-coated zinc.[3] Connect the flask to a distilling apparatus and arrange the exit of the condenser tube with a glass tube passing to the bottom of a 100 c.c. flask in which has been placed 1 or 2 c.c. of distilled water and a single drop of hydrochloric acid. Now heat the flask gently for one hour (do not boil) and then distil the contents until nearly the whole has evaporated; then fill up the funnel with hot distilled water and turn on the stop-cock, allowing the whole to pass over into the 100 c.c. flask. Repeat addition of water to the flask and continue distillation until about 90 c.c. have been collected. Remove the 100 c.c. flask,

[1] *Jnl. Chem. Soc.*, 1878, 140. [2] *Ibid.*, 1881, 100.

[3] Granulated zinc is immersed in strong copper sulphate solution until metallic copper is deposited on the surface of the zinc.

dilute to mark with distilled water free from ammonia, agitate, take 5 c.c., dilute to 50 c.c., and Nesslerise as explained (p. 169).

Each equivalent of ammonia found corresponds to one equivalent of nitric acid; that is to say, each 17 parts of ammonia correspond to 54 parts of nitric acid;

$$\frac{54}{17} = 3{\cdot}176.$$

So that by multiplying the ammonia found by the factor 3·176, we get the nitric acid, and the result is calculated to grains per gallon.

Example.—250 c.c. of water taken and 5 c.c. of the distillate (after diluting to 50 c.c.) required 7·0 c.c. of standard ammonia to produce equivalent colour value. $7{\cdot}0 \times {\cdot}01 = {\cdot}070$ milligram of NH_3 in the 5 c.c. or 1·40 in the whole distillate containing the ammonia from the nitric acid in 250 c.c. of the water. Then $1{\cdot}40 \times {\cdot}4 = {\cdot}560$ milligrams of NH_3 in 100 c.c. $\times {\cdot}7 = {\cdot}3920$ in 70 c.c., *i.e.* ·3920 grain per gallon NH_3 due to nitric acid. This multiplied by $3{\cdot}176 =$ **1·25 grain per gallon nitric anhydride.**

12. **Nitrous Acid: Greiss's Method of Detection.**[1]—It is only in very impure waters that nitrous acid is found, and even in these cases it generally exists in quantity too minute for quantitative estimation.

The author has never yet found nitrous acid in a deep well water, and believes that where it has been detected in such waters it is due more to the system adopted than to the fact of its existence.

The simplest method of detection is that proposed by Greiss, which consists in the addition of meta-diamido-benzene to the water after acidifying, which imparts a yellow colour with the most minute traces of nitrous acid. 100 c.c. of the water are taken in a Nessler tube, 1 c.c. of strong sulphuric acid added, and then a few drops of meta-diamido-benzene solution. The tube, after standing for a few minutes, is then examined by looking down through it, on a white porcelain tile or piece of white paper.

Nitrous Acid: Fresenius's Method of Estimating.—The following method is given by Fresenius[2] for the quantitative

[1] For full particulars of Greiss's method, see paper by R. Warrington, *Jnl. Chem. Soc.*, 1881, 229.

[2] *Fresenius's Quantitative Analysis*, Churchill.

estimation should nitrites be present in quantity in a water, which is seldom the case:—

Take 250 c.c. of the water at 60° F. (15·5° C.), acidify by the addition of a few drops of acetic acid, place in a retort and distil off about 100 c.c., collecting the same in a 250 c.c. flask and making up the distillate to mark with distilled water.

A portion of this distillate is then taken, acidified with a few drops of pure sulphuric acid, and titrated against a very weak solution of potassic permanganate, 30 c.c. of which should equal ·01 gram of iron = ·0034 nitrous anhydride N_2O_3.

Organic Matter.—We are indebted to Wanklyn, Chapman, and Smith for the now well-known "albuminoid ammonia process" of estimating the quantity of nitrogenous organic matter in water, which dates from the year 1867.[1]

The method depends upon the conversion of the nitrogen in such organic matter into ammonia when distilled with an alkaline solution of potassic permanganate. The authors have given the term "albuminoid ammonia" to the NH_3 produced from nitrogenous matter by the action of permanganate, doubtless because the first experiments made in the process were made with albumin; but they also proved that ammonia may be obtained in a similar manner from a great variety of nitrogenous organic substances, such as hippuric acid, narcotine, strychnine, morphine, creatine, gelatine, casein, etc. Unfortunately, however, although the proportion of nitrogen yielded by one substance when treated with boiling alkaline permanganate appears to be definite, yet different substances give different proportions of their nitrogen. Thus hippuric acid and narcotine yield the whole, but strychnine and morphine only one-half of their known proportions of nitrogen. Hence the value of the numerical results thus obtained depends entirely on the assumption that the nitrogenous organic matter in water is *uniform in its nature*, and the authors say that in a river polluted mainly by sewage "the disintegrating animal refuse would be pretty fairly measured by ten times the albuminoid ammonia which it yields."

They also state that "the albuminoid ammonia from a really good drinking water should not exceed 0·008 parts per 100,000." The average of fifteen samples of Thames water supplied to London by the various Water Companies some years ago was 0·0089, and in five samples supplied by the New River Company 0·0068 parts per 100,000. The rapidity and simplicity of the operation are the chief merits of this process, and the information

[1] *Jnl. Chem. Soc. N.S.*, vol. v. 591.

to be obtained from its performance may, for some purposes, be of considerable value; and even if the numerical results cannot be insisted upon, yet *a good water could not be condemned by it, and a bad one should not escape its indications.*

There are two estimations to be made, viz. :—

13. Free or saline ammonia.
14. Albuminoid ammonia.

13. **Estimation of Free or Saline Ammonia.**—Half a litre (500 c.c.) of the water at 60° F. (15·5° C.) is measured and added to a retort, together with 10 c.c. of a saturated solution of sodic carbonate. The retort is then connected with a Liebig distilling apparatus (fig. 35, p. 31), condensing water turned on, and a Bunsen-flame placed close under the bottom of the retort and lighted full on. The distillation soon commences, and the distillate should be received in a tall glass cylinder of about 150 c.c. capacity, and marked at 50 c.c. and 100 c.c.

Four such cylinders are required at hand during the operation.

When 50 c.c. have distilled over, the cylinder should be removed and a fresh cylinder placed to receive a further 50 c.c. of the distillate.

Whilst this second cylinder is slowly receiving the second 50 c.c. distillate, the free or saline ammonia contained in the first 50 c.c. should be tested.[1]

In order to perform this, a standard tube is first made up as follows:—

Nesslerising.

To a tall glass cylinder of a capacity of 150 c.c., marked at 50 c.c. and 100 c.c., similar to those employed for the collection of the distillate, but graduated at each 5 c.c., is added from a burette 5 c.c. of standard ammonia (p. 139), and then made up to 50 c.c. with water free from ammonia (p. 139)[2] and mixed. One c.c. of Nessler's solution (p. 139) is then added, and the whole mixed. One c.c. of Nessler's solution is at the same time added to the 50 c.c. distillate, and both this tube and the

[1] It is inadvisable to allow the tubes containing the distillate to remain standing in the laboratory for any length of time, as (water being very absorbent) ammoniacal or sulphuretted hydrogen fumes may be absorbed and thus vitiate the results

[2] Distilled water invariably contains a large percentage of ammonia, and therefore, if not previously freed from the same, should not be used. Good tap-water will usually be found free, and may, therefore, in most cases be employed.

standard tube are now placed on a white surface, such as a white tile, and allowed to stand for 5 minutes. By this time a yellow or brown colour will be produced in the distillate, varying in intensity with the amount of free ammonia contained in the water, and the object is to imitate the colour in the standard tube.

The two tubes are therefore taken and examined side by side. If the colour in the standard tube is too deep, withdraw sufficient solution, so that, on looking down the tubes on to the white surface, the colours are exactly of the same intensity. Note the amount of standard ammonia required. If the colour is darker than that of the standard, a fresh standard of double strength should be made. The second 50 c.c. distillate is now taken and Nesslerised in a similar manner; a third 50 c.c. distillate collected and Nesslerised, and again a fourth 50 c.c. if necessary. Three separate 50 c.c.'s, however, usually suffice with most waters. Generally speaking, the whole of the free NH_3 will come over in the first 100 or 150 c.c., but the distillation should be continued until 50 c.c. of the distillate contain less than $\frac{1}{100}$ milligram of NH_3.

Wanklyn has recommended the uniform plan of Nesslerising only the first 50 c.c. for free ammonia, throwing away the 150 c.c. subsequently distilled, and calculating that it contains one-third of the quantity found in the first 50 c.c.

14. **Estimation of Albuminoid Ammonia.**—To the contents of the retort left from the operation just described, are at once added, through a clean funnel inserted into the tubulure of the retort, 50 c.c. alkaline permanganate (p. 140). Having added this, resume the distillation, and estimate the ammonia as before until no more is evolved; generally speaking, it is sufficient to distil 150 c.c. after adding the alkaline permanganate, estimating the ammonia in each 50 c.c. The boiling is often very irregular, especially in bad waters, and if so it is advisable to introduce into the retort a few small pieces of freshly ignited pumice or pipe-clay to moderate the bumping. It is always advisable to incline the neck of the retort upwards, so that the liquid carried up by spirting may be returned; especially as manganese compounds in particular have a powerful effect upon the colour produced by the Nessler solution, generally intensifying it, and thus vitiating results. The amount of ammonia estimated by the Nessler test in this distillate is entered as albuminoid ammonia. The number of c.c. of standard ammonia required to imitate the colour intensity in each tube are now added together, and

the result calculated and expressed in parts per million as follows:—

The graduated tube contained 5 c.c. of standard ammonia (0·01 milligram per c.c.), 50 c.c. therefore contain 0·05 milligram of ammonia.

Free Ammonia—

1st tube, 50 c.c. distillate from $NaCO_3$	=	42 c.c. standard ammonia.
2nd tube, 50 c.c. distillate from $NaCO_3$	=	19 „ „
3rd tube, 50 c.c. distillate from $NaCO_3$	=	Nil.
		61 c.c. total to equal tint.

Now 50 c.c. = ·05 milligram of ammonia, therefore ·061 milligram is present in the 61 c.c.; that is, in 500 c.c. of the water, or ·122 per 1000 c.c. of water. Now 1000 c.c. contain 1,000,000 milligrams, therefore milligrams per 1000 c.c. = parts per million.

The sample therefore contains **0·122 parts per million of free ammonia.**

Albuminoid Ammonia (calculated in the same way):—

1st tube, 50 c.c. distillate from potassic permanganate	=	19 c.c. standard ammonia.
2nd tube, 50 c.c. distillate from potassic permanganate	=	12 „ „
3rd tube, 50 c.c. distillate from potassic permanganate	=	Nil.
		31 c.c. total.

31 c.c. = ·031 milligram of ammonia in 500 c.c., or **0·062 milligram in 1000 c.c. or parts per million.**

If it is desired to express the result in parts per 100,000, instead of in parts per million, it is written thus:—

Free ammonia = 0·012.
Albuminoid ammonia = 0·006.

15. **Oxygen required to oxidise Organic Matter.**—This process, originally designed by Forschammer and reintroduced by Tidy, depends upon the estimation of the amount of oxygen absorbed in oxidising the organic and other oxidisable matters in

a known volume of water, when slightly acidified with sulphuric acid.

For this purpose a standard solution of potassic permanganate (p. 138) is used in excess; the amount unoxidised after a given time being ascertained by the help of a standard solution of sodic thiosulphate (p. 138), by the aid of the well-known iodide of starch reaction.

Ferrous salts, nitrites, or sulphuretted hydrogen, if present in the water, also decompose the permanganate; but as organic matters are only slowly oxidised, the bodies named affect the permanganate almost immediately. Therefore, by conducting two experiments, one after standing 15 minutes, the other after standing 4 hours, the reduction of the permanganate due to iron, nitrites, etc., and that due to organic matter, may be determined.

The estimation is performed as follows:—

A stoppered bottle of about 300 c.c. capacity is carefully rinsed out with dilute sulphuric acid and well rinsed afterwards with distilled water. Ten c.c. of standard potassic permanganate and 200 c.c. of the water are then added, and afterwards 10 c.c. of dilute sulphuric acid, which is first rendered a faint pink by the addition of a few drops of the permanganate.

The bottle, with the stopper replaced, is then set aside on the forcing tray and maintained at about 75°–80° F. (23·8°–26·6° C.) for 4 hours.

At the end of this time, the undecomposed permanganate is determined as follows:—

One or two drops of potassic iodide solution are added to the water in the bottle, which change the pink colour to yellow, due to the liberation of the free iodine. Standard thiosulphate solution is now run in from a burette until the yellow colour has nearly, but not quite, disappeared. A few drops of freshly prepared gelatinised starch solution [1] are now added, which turn the solution blue. The thiosulphate is then again added to the solution until the blue colour is just destroyed. The quantity of thiosulphate used is noted. Let us denote this as A.

As the thiosulphate solution is liable to slightly change upon keeping, a blank experiment is now made to determine its value. This is performed by taking 200 c.c. distilled water, 10 c.c. of the standard permanganate, and 10 c.c. of dilute sulphuric acid made faintly pink with permanganate. A drop of potassic iodide is

[1] Gelatinised starch, prepared by mixing about 1 gram of potato starch with about 80 c.c. distilled water, boiling for a few minutes, cooling, and making up to about 100 c.c.

added, which changes the colour to yellow, and the solution is again titrated with the thiosulphate solution. This gives the value of the thiosulphate solution. Let us denote this as B.

A further experiment is now performed with a further 200 c.c. of the water, with the addition of permanganate and acid as before, but allowed to stand 15 minutes, after which a drop of potassic iodide is added and the solution titrated wit thiosulphate as before described. Let us denote this by C.

From the results of the three experiments the amount of oxygen absorbed is thus obtained :—

A. 200 c.c. of water after 4 hours required	9·3 c.c. thiosulphate solution.	
C. 200 c.c. of water after 15 minutes required	10·8 ,,	,,
B. Blank experiment required	11·4 ,,	,,

11·4 equals 10 c.c. permanganate, therefore 9·3 = 8·1

$$\frac{10 \times 9{\cdot}3}{11{\cdot}4} = 8{\cdot}1$$

and $10 - 8{\cdot}1 = 1{\cdot}9$ permangante absorbed in 4 hours.

Similarly, as 11·4 equals 10 c.c. permanganate, 10·8 equals 9·5, and $10 - 9{\cdot}5 = 0{\cdot}5$ permanganate decomposed in 15 minutes.

Each c.c. of the standard permanganate equals 0·1 milligram of available oxygen, so that the above figures correspond to ·19 and ·05 milligram respectively per 100 c.c. of water.

It is usual, however, to express the results in terms of parts per 100,000, so that as 100 c.c. contains 100,000 milligrams, we get :—

Oxygen absorbed in 15 minutes . . .	·025
Oxygen ,, 4 hours . . .	·095

16. **Silica**.—It is only in exceptional circumstances that the estimation of silica is necessary; such, for instance, as when the water is seen to contain an excess of suspended or deposited matter. In such instances the estimation is carried out as follows :—

To 500 c.c. of the water at 60° F. (15·5° C.) add a few drops of hydrochloric acid, and evaporate by degrees to dryness in a platinum dish. Heat to 300° F. (148·8° C.), cool, add a few more drops of hydrochloric acid and about 50 c.c. distilled water. Stir the residue by the aid of a rubber-tipped glass rod and allow to stand when the silica precipitates.

Filter, wash the precipitate with distilled water, dry the filter

paper and its contents in the water bath, ignite, cool under desiccator, and weigh.

Example:—

Weight of platinum dish + SiO_2	9·688
Weight of platinum dish	9·685
	·003

$$\cdot 0030 \times \cdot 4 = \cdot 00120 \times \cdot 7 = \cdot 00084.$$
$$= 0{\cdot}84 \text{ grain per gallon.}$$

17. **Lead.**—Add 50 c.c. of the water at 60° F. (15·5° C.) to a Nessler tube, acidulate with a few drops of acetic acid, and then run in 10 c.c. of saturated aqueous solution of sulphuretted hydrogen.

Compare the colour thus produced in a similar cylinder with a known quantity of a standard solution of lead acetate in a manner similar to that described for the estimation of iron.

The lead solution may be prepared so as to contain 0·183 gram of normal crystallised plumbic acetate in a litre of distilled water at 60° F. (15·5° C.); each c.c. will therefore correspond to ·001 gram of metallic lead.

18. **Copper.**—Of the coloured reactions which copper gives with different reagents, those with sulphuretted hydrogen and potassic ferrocyanide are by far the most delicate, both showing their respective colours in 2,500,000 parts of water. Of the two reagents sulphuretted hydrogen is the more delicate; but potassic ferrocyanide has a decided advantage over sulphuretted hydrogen inasmuch as lead, when not present in too large quantity, does not interfere with the depth of colour obtained, whereas to sulphuretted hydrogen it is, as is well known, very sensitive. And though iron if present would, without special precautions being taken, prevent the determination of copper by means of potassic ferrocyanide, yet, by the method here described, the copper in a solution can be estimated by this reagent. Ammonic nitrate renders the reaction much more delicate; other salts, such as ammonic chloride and potassic nitrate, have likewise the same effect.

The method of analysis consists in the comparison of the purple-brown colours produced by adding to a solution of potassic ferrocyanide—first, a solution of copper of known strength; and secondly, the solution in which the copper is to be determined.

To 100 c.c. of the water at 60° F. (15·5° C.) add two or three drops of nitric acid and evaporate to dryness in a platinum dish.

Ignite the residue to get rid of any organic matter that may colour the liquid, and dissolve in a little boiling distilled water and a drop or two of nitric acid; if the residue is not all soluble, it does not matter. Ammonia is next added to precipitate the iron, the latter filtered off, washed, redissolved in nitric acid, and again precipitated by ammonia, filtered off, and washed. The filtrate is added to the one previously obtained, and the copper estimated in the united filtrates after rendering the liquid neutral.

The liquid must be neutral, for, if it contain free acid, the latter lessens the depth of colour and changes it from a purple-brown to an earthy brown. If it should be acid, it is rendered slightly alkaline with ammonia, and the excess of the latter got rid of by boiling. The solution must not be alkaline, as the brown coloration is soluble in ammonia and decomposed by potash or soda. If it be alkaline from ammonia, this is removed by boiling; while free potash or soda, should they be present, are neutralised by an acid and the latter by ammonia.

Example.—Two Nessler tubes are taken and into each is run five drops of potassic ferrocyanide. Fifty c.c. of the neutral water to be tested are now run into one cylinder and a similar quantity of distilled water into the other; 5 c.c. of ammonic nitrate, 10 per cent. solution, are now added to each cylinder, and then the colour produced in the cylinder containing the water under examination is imitated by running into the other cylinder standard copper solution till the colours in both cylinders are of equal depth, the liquid being well stirred after each addition of the copper solution.

Combination of Acids and Bases.—The amount of acids and bases having been determined, it now becomes necessary to calculate the salts in the form in which they are generally believed to exist in water. The salts are usually calculated on the basis proposed some years ago by Fresenius, the general rules laid down by him being as follows:—

(1) The chlorine is combined with sodium.

(2) Any chlorine afterwards left is combined with calcium, magnesium, and potassium in respective order.

(3) Potash is next combined with sulphuric acid.

(4) If sodium remains, it is combined with nitric acid (N_2O_5); and if there is still an excess, it is combined with carbon-dioxide.

(5) Lime is first combined with nitric acid, then with sulphuric acid, and finally with carbon-dioxide. Whilst if chlorine is in excess of that required to saturate the sodium (these amounts generally saturate one another), it is combined with calcium.

(6) Magnesia is combined first with nitric acid (if lime has not saturated it) then with sulphuric acid, whilst any excess is combined with carbon-dioxide.

(7) Any excess of lime is always finally combined with carbon-dioxide; the reason being that any ordinary water contains CO_2, and any excess of lime must combine with the same to form carbonates.

The sum of these constituents is checked by the alkalinity of the water, so that if, by calculation, carbonate of potash or soda is found to exist, the alkalinity of the water after boiling would prove the existence of one or the other or both.

The total salts should almost equal the saline residue of the water when dried at 350° F. (176·6° C.), provided nitrates are not present.

We may now proceed to combine the acids and bases of the foregoing analysis, which is that of a water drawn from a well at Burton-on-Trent, suitable only for bitter-ale production, and follow with typical combinations of a water suitable for stout and porter, and then one suitable for mild-ale production. First, however, it may be useful if we tabulate the proportions of the various acids and bases necessary to saturate one another in order to form salts.

80 parts sulphuric acid combine with
- 56 parts lime to form sulphate of lime.
- 40 ,, magnesia to form sulphate of magnesia.
- 62 ,, soda to form sulphate of soda.
- 94 ,, potash to form sulphate of potash.

108 parts nitric acid combine with
- 56 parts lime to form nitrate of lime.
- 40 ,, magnesia to form nitrate of magnesia.
- 62 ,, soda to form nitrate of soda.

44 parts carbonic acid combine with
- 56 parts lime to form carbonate of lime.
- 40 ,, magnesia to form carbonate of magnesia.
- 62 ,, soda to form carbonate of soda.

71 parts chlorine (35·5 × 2) combine with
- 40 parts calcium to form calcic chloride.
- 24 ,, magnesia to form magnesic chloride.
- 46 ,, soda to form sodic chloride.

Now, taking the results of our previously conducted analysis, we proceed to combine the acids and bases.

TYPE I.

The sodium being in excess, we take the chlorine and combine it with sodium:—

35·5 parts Cl require 23 parts Na.

Therefore $2{\cdot}37\ Cl \times \frac{23}{35{\cdot}5} = 1{\cdot}53\ Na$ required,

and $1{\cdot}53\ Na + 2{\cdot}37\ Cl = \mathbf{3{\cdot}90\ NaCl}$.

We now take the potash and combine it with sulphuric acid:—

94 parts K_2O require 80 SO_3.

Therefore $0{\cdot}86\ K_2O \times \frac{80}{94} = 0{\cdot}73\ SO_3$ required,

and $0{\cdot}73\ SO_3 + 0{\cdot}86\ K_2O = \mathbf{1{\cdot}59\ K_2SO_4}$.

We have now to combine nitric acid with sodium:—

108 parts Na_2O combine with 62 parts N_2O_5.

Therefore $1{\cdot}25\ N_2O_5 \times \frac{62}{108} = 0{\cdot}71\ Na_2O$ required,

and $0{\cdot}71\ Na_2O + 1{\cdot}25\ N_2O_5 = \mathbf{1{\cdot}96\ Na(NO_3)_2}$.

Thus far we have used up 1·53 Na to form NaCl, and 0·71 Na_2O to form sodic nitrate. But in our analytical figures the soda 7·25 is expressed as Na_2O; we have therefore to calculate the 1·53 Na back to Na_2O:—

46 parts Na require 62 parts Na_2O.

So $1{\cdot}53$ (amount Na used) $\times \frac{62}{46} = 2{\cdot}06\ Na_2O$.

Therefore $2{\cdot}06\ Na_2O + 0{\cdot}71\ Na_2O = 2{\cdot}77$, the amount of Na_2O used,
and $7{\cdot}25\ Na_2O - 2{\cdot}77 = 4{\cdot}48\ Na_2O$ required.

We have now to combine this 4·48 Na_2O with SO_3:—

62 parts Na_2O require 80 parts SO_3.

$4{\cdot}48$ (Na_2O remaining) $\times \frac{80}{62} = 5{\cdot}78\ SO_3$ required,

and $5{\cdot}78 + 4{\cdot}48 = \mathbf{10{\cdot}26\ Na_2SO_4}$.

We have thus used up our soda, 0·73 SO_3 for K_2SO_4 and 5·78 SO_3 for Na_2SO_4 (= 6·51 SO_3), leaving (52·29 − 6·51) 45·78 SO_3.

We have now to combine the SO_3 with lime (CaO):—

80 parts SO_3 require 56 CaO.

Therefore $45·78$ (remaining SO_3) $\times \frac{56}{80} = 32·04$ CaO required,

and $32·04\ CaO + 45·78\ SO_3 = \mathbf{77·82\ CaSO_4}$.

This disposes of our SO_3 and leaves us with $(36·33 - 32·04) = 4·29$ CaO :—

56 parts CaO require 44 parts CO_2.

Therefore $4·29$ (remaining CaO) $\times \frac{44}{56} = 3·37\ CO_2$,

and $4·29\ CaO + 3·37\ CO_2 = \mathbf{7·66\ CaCO_3}$.

(There are no acids left to combine with the magnesia, which must therefore exist as carbonate) :—

40 parts MgO require 44 parts CO_2.

Therefore $10·15$ (amount of MgO) $\times \frac{44}{40} = 11·16\ CO_2$,

and $10·15\ M_9O + 11·16\ CO_2 = \mathbf{21·31\ MgCO_3}$.

We have already estimated the alkalinity of the water both before and after boiling, and seen that after boiling the alkalinity is equal to 31·80 grains per gallon $CaCO_3$; the amount of carbonates arrived at by calculation may therefore be checked by this figure.

In our combination results we have 21·31 grains per gallon carbonate of magnesia :—

88 parts $MgCO_3$ have the same alkalinity as 100 parts $CaCO_3$.

So $21·31 \times \frac{100}{88} = 24·21$ as $CaCO_3$.

Therefore actual $CaCO_3$ $7·66 + 24·21 = 31·87$ grains of alkalinity, and by our test 31·80 grains.

The composition of the water is therefore as follows :—

TYPE I.

Total solids	139·920	grains	per gallon.
Saline residue . . .	125·400	,,	,,
Organic and volatile matter .	14·520	,,	,,
Lime	36·330	,,	,,
Magnesia	10·150	,,	,,
Sulphuric acid . . .	52·290	,,	,,
Chlorine	2·380	,,	,,
Iron	0·098	,,	,,
Soda	7·250	,,	,,
Potash	0·860	,,	,,
Silica	0·840	,,	,,

Nitrogen as nitrates and nitrites :—	
Nitric acid	1·250 grains per gallon.
Nitrous acid . . .	Nil.
Organic matter :—	
Free or saline ammonia .	0·122 parts per million.
Albuminoid ammonia . .	0·062 ,, ,,
Oxygen required to oxidise organic matter :—	
15 minutes . . .	0·025 parts per 100,000
4 hours	0·095 ,, ,,

MOST PROBABLE COMBINATION.

Sodium chloride ($NaCl$) . .	3·90 grains per gallon.
Potassium sulphate (K_2SO_4) .	1·59 ,, ,,
Sodium nitrate $Na(NO_3)_2$ . .	1·96 ,, ,,
Sodium sulphate (Na_2SO_4) . .	10·26 ,, ,,
Calcium sulphate ($CaSO_4$) . .	77·82 ,, ,,
Calcium carbonate ($CaCO_3$) .	7·66 ,, ,,
Magnesium carbonate ($MgCO_3$) .	21·31 ,, ,,
Iron oxide (Fe_2O_3) . . .	0·09 ,, ,,
Silica (SiO_3)	0·84 ,, ,,
Saline residue . .	125·43 ,, ,,

Saline residue found = 125·40 grains per gallon.

TYPE II.

We will now take a typical analysis of a water suitable for mild ale production, the analytical figures of which have been determined as follows :—

Silica	0·26 grains per gallon.
Iron oxide . . .	0·24 ,, ,,
Lime	9·79 ,, ,,
Magnesia . . .	0·43 ,, ,,
Soda	0·97 ,, ,,
Chlorine . . .	1·11 ,, ,,
Sulphuric acid . .	2·62 ,, ,,

We first take the sodium ($Na_2O = 0{\cdot}97$) and calculate it back to Na :—

62 parts Na_2O contain 46 Na.

Therefore $\quad 0{\cdot}97 \text{ (amount of } Na_2O) \times \frac{46}{62} = 0{\cdot}71$ Na.

We combine this with chlorine :—

23 parts Na require 35·5 parts Cl.

So $\quad 0{\cdot}71 \text{ (amount of Na)} \times \frac{35{\cdot}5}{23} = 1{\cdot}11$ Cl required,

and $\quad 0{\cdot}71 \text{ Na} + 1{\cdot}11 \text{ Cl} = \mathbf{1{\cdot}82\ NaCl}.$

This disposes of our soda and chlorine (these amounts just saturating one another). We have neither potash nor nitric acid, so we proceed to combine sulphuric acid (SO_3) with lime (CaO):—

80 parts SO_3 require 56 parts CaO.

Therefore $\quad 2{\cdot}62 \text{ } SO_3 \times \frac{56}{80} = 1{\cdot}83$ CaO required,

and $\quad 2{\cdot}62 \text{ } SO_3 + 1{\cdot}83 \text{ CaO} = \mathbf{4{\cdot}45\ CaSO_4}.$

Our CaO equalled 9·79, and as we have used 1·83 parts of it to form $CaSO_4$, we have 7·96 left, which we combine with CO_2:—

56 parts CaO require 44 parts CO_2.

Therefore $\quad 7{\cdot}96 \text{ (remaining CaO)} \times \frac{44}{56} = 6{\cdot}25 \text{ } CO_2,$

and $\quad 6{\cdot}25 \text{ } CO_2 + 7{\cdot}96 \text{ CaO} = \mathbf{14{\cdot}21\ CaCO_3}.$

We next combine magnesia (0·43) MgO with CO_2:—

40 parts MgO require 44 parts CO_2.

Therefore $\quad 0{\cdot}43 \text{ (amount of MgO)} \times \frac{44}{40} = 0{\cdot}47 \text{ } CO_2.$

and $\quad 0{\cdot}43 \text{ MgO} + 0{\cdot}47 \text{ } CO_2 = \mathbf{0{\cdot}90\ MgCO_3}.$

The composition of the water is therefore:—

Sodium chloride (NaCl)	1·82	grains	per gallon.
Calcium sulphate ($CaSO_4$)	4·45	,,	,,
Calcium carbonate ($CaCO_3$)	14·21	,,	,,
Magnesium carbonate ($MgCO_3$)	0·90	,,	,,
Iron oxide (Fe_2O_3)	0·24	,,	,,
Silica (SiO_3)	0·26	,,	,,

Type III.

We finally take a typical analysis of a water suitable for stout and porter production, the analytical figures of which have been determined as follows:—

Silica	0·22	grains	per gallon.
Iron	0·24	,,	,,
Lime	13·13	,,	,,
Magnesia	1·91	,,	,,
Soda	18·62	,,	,,
Chlorine	23·81	,,	,,
Sulphuric acid	3·67	,,	,,

We first take the soda, which is expressed in our analysis as Na_2O, and calculate it back to Na:—

$$62 \text{ parts } Na_2O \text{ contain } 46 \text{ parts Na.}$$

$$18{\cdot}62 \text{ (amount of } Na_2O) \times \frac{46}{62} = 13{\cdot}81 \text{ Na.}$$

We now combine this with chlorine :—

$$23 \text{ parts Na require } 35{\cdot}5 \text{ Cl.}$$

Therefore $\quad 13{\cdot}81 \text{ Na} \times \frac{35{\cdot}5}{23} = 21{\cdot}31 \text{ Cl required,}$

and $\quad 21{\cdot}31 \text{ Cl} + 13{\cdot}81 \text{ Na} = \mathbf{35{\cdot}12\ NaCl}.$

We have thus used up our sodium and have ($23{\cdot}81$ Cl − $21{\cdot}31$ Cl) $2{\cdot}50$ Cl left.

We have now to combine this Cl with calcium (Ca) :—

$35{\cdot}5$ parts Cl × 2 = 71 parts Cl, which combine with 40 parts Ca to form CaCl.

Therefore $\quad 2{\cdot}50 \text{ (Cl remaining)} \times \frac{40}{71} = 1{\cdot}40 \text{ Ca required,}$

and $\quad 1{\cdot}40 \text{ Ca} + 2{\cdot}50 \text{ Cl} = \mathbf{3{\cdot}90\ CaCl}.$

We now take our SO_3 ($3{\cdot}67$) and combine it with lime (CaO) :—

$$80 \text{ parts } SO_3 \text{ require } 56 \text{ parts CaO.}$$

Therefore $\quad 3{\cdot}67\ (SO_3) \times \frac{56}{80} = 2{\cdot}56 \text{ CaO required,}$

and $\quad 2{\cdot}56 \text{ CaO} + 3{\cdot}67\ SO_3 = \mathbf{6{\cdot}23\ CaSo_4}.$

Of our original lime $13{\cdot}13$ we have bound $1{\cdot}40$ with chlorine to form CaCl and $2{\cdot}56$ with SO_3 to form $CaSO_4$: $1{\cdot}40 + 2{\cdot}56 = 3{\cdot}96$ and $13{\cdot}13 - 3{\cdot}96 = 9{\cdot}17$.

Lime remaining :—

$$56 \text{ parts CaO require } 44 \text{ parts } CO_2.$$

Therefore $\quad 9{\cdot}17 \text{ (lime remaining)} \times \frac{44}{56} = 7{\cdot}20\ CO_2,$

and $\quad 9{\cdot}17 \text{ (lime)} + 7{\cdot}20\ CO_2 = \mathbf{16{\cdot}37\ CaCO_3}.$

We now finish with the magnesia ($1{\cdot}91$ MgO) :—

$$40 \text{ parts MgO require } 44 \text{ parts } CO_2.$$

Therefore $\quad 1{\cdot}91 \text{ (amount of MgO)} \times \frac{44}{40} = 2{\cdot}10\ CO_2,$

and $\quad 1{\cdot}91 \text{ MgO} + 2{\cdot}10\ CO_2 = \mathbf{4{\cdot}01\ MgCO_3}.$

The composition of the water is therefore :—

Sodium chloride ($NaCl$) . .	35·12	grains per gallon.
Calcium chloride ($CaCl_2$) . .	3·90	,, ,,
Calcium sulphate ($CaSO_4$) . .	6·23	,, ,,
Calcium carbonate ($CaCO_3$) .	16·37	,, ,,
Magnesium carbonate ($MgCO_3$) .	4·01	,, ,,
Iron oxide (Fe_2O_3)	0·24	,, ,,
Silica (SiO_3)	0·22	,, ,,

The foregoing calculations can all naturally be shortened by cancelling and the use of factors, but are given in detail so as to be explicit to the student.

Bisulphite of Lime Analysis.

The following are the determinations to be made :—

1. Specific gravity.
2. Total sulphurous acid (free and combined).
3. Sulphuric acid.
4. Lime.
5. Magnesia.
6. Chlorine.
7. Iron.
8. Hyposulphites.

The bisulphite should be drawn from the cask through a freshly bored peg-hole into a clean dry stoppered bottle, the stopper being tightly fitted and the contents well shaken before use. The bottle should be immersed in cold water or the contents otherwise brought to 60°F. (15·5° C.) before commencing the analysis, and owing to the rapidity with which the sulphite oxidises when exposed to the air and the volatilisation of SO_2, it is essential to perform each experiment as quickly as possible.

1. **Specific Gravity.** — This is determined in the ordinary manner by the aid of the specific-gravity bottle (fig. 36, p. 33).

2. **Total Sulphurous Acid.** *Gravimetric Method.*—The total sulphurous acid may be determined either gravimetrically or volumetrically. In the former case the sulphurous acid, both free and combined, is first oxidised into sulphuric acid and then determined by the aid of baric chloride, the amount of sulphurous acid being calculated from the weight of the precipitated baric sulphate. We have first, however, to determine the weight of baric sulphate

which may be due to any sulphuric acid existing as such in the bisulphite owing to oxidation, and deduct this from the total weight of baric sulphate, calculating the difference into sulphurous acid. We therefore proceed as follows:—

Add to a beaker about 120 c.c. distilled water and about 10 c.c. bromine and stir well. Now measure 20 c.c. of the bisulphite in a 200 c.c. flask and make to mark with distilled water = 10 per cent. solution. Run 10 c.c. of this 10 per cent. solution into the previously prepared bromine water = 1 c.c. of bisulphite. The solution should now be distinctly brown; if it is not, add a few more drops of bromine and stir well. Now add 2 or 3 c.c. hydrochloric acid and boil the solution over a Bunsen flame until the whole of the free bromine is volatilised and the solution is free from colour. The sulphurous acid has now been oxidised into sulphuric acid, the bromine having decomposed the elements of water and combined with the hydrogen to form hydrobromic acid, whilst the sulphurous acid has seized the liberated oxygen.

The sulphuric acid is now determined in the solution in exactly the same manner as in water analysis, and the sulphurous acid calculated after deducting the $BaSO_4$ due to sulphuric acid naturally present in the sample.

Example.—To the colourless boiled solution a few drops of baric chloride are added, the solution further boiled for a minute or two and then set aside in a warm place until the baric sulphate has settled and the supernatant liquid is perfectly clear. The solution is then filtered, the filter paper repeatedly washed with hot distilled water, dried, folded, and ignited in a tared crucible. The crucible is then cooled under the desiccator and weighed.

The weight is found, say, to be:—

Weight of crucible + $BaSO_4$ . . .	6·801 grams.
Weight of crucible	6·540 „
$BaSO_4$ . . .	0·261

It is found, however, that 1 c.c. of the bisulphite contains ·007 $BaSO_4$ due to sulphates naturally present, so $0·261 - 0·007 = 0·254$ $BaSO_4$ from sulphurous acid (SO_2) in 1 c.c.

As shown under Water Analysis, the atomic weight of baric sulphate is 233, and that of sulphurous anhydride 64, so that every 233 parts of $BaSO_4$ correspond to 64 parts SO_2, or 1 gram to $0·2746 = 0·06974$ gram SO_2 in 1 c.c. bisulphite or 6·974 per 100 c.c.

Let us now confirm this gravimetric method by the *volumetric process.*

This depends upon the beautiful and sensitive iodine reaction already fully described under Sulphurous Acid (p. 149).

Measure 20 c.c. of the bisulphite into a 200 c.c. flask and dilute to mark with recently prepared distilled water. Now take 10 c.c. of this solution in a porcelain dish, add a few drops of gelatinised starch solution (footnote, p. 172), and titrate with $\frac{N}{10}$ iodine until a blue colour makes its appearance and becomes permanent on standing.

Suppose the amount of $\frac{N}{10}$ iodine used = 21 c.c. Then, as each c.c. of iodine corresponds to 0·0032 sulphurous acid $21 \times 0{\cdot}0032 = 0{\cdot}0672$ SO_2 in 10 c.c. dilute bisulphite (= 1 c.c. of bisulphite), and $0{\cdot}0672 \times 100 = 6{\cdot}72$ SO_2 per 100 c.c. as against 6·97 per 100 c.c. found gravimetrically. If only the volumetric method is employed, then it becomes necessary to confirm the result by titration with $\frac{N}{10}$ iodine, by using an excess of the latter and performing a second titration by the use of $\frac{N}{10}$ thiosulphate as exemplified under Sulphurous Acid, p. 149.

3. **Sulphuric Acid.**—Add about 10 c.c. concentrated hydrochloric acid and about 100 c.c. distilled water to a small boiling flask and boil for a few minutes to expel the air from the flask and free oxygen from the water. Now add 50 c.c. of the bisulphite and boil for about 15 minutes, or until the evolved steam no longer smells of sulphurous acid. Transfer the contents of the flask to a beaker, dilute slightly with distilled water, add about 10 c.c. baric chloride solution, and set aside in a warm place until the baric sulphate has precipitated and the supernatant liquid is clear. Now filter, wash with hot distilled water, dry, ignite, cool, and weigh as baric sulphate ($BaSO_4$).

Example :—

Weight of crucible + ash	6·797 grams.
Weight of crucible	6·500 ,,
$BaSO_4$	0·297

The atomic weight of $BaSO_4$ is 233, and that of sulphuric anhydride (SO_3) 80. 233 parts of $BaSO_4$ therefore correspond to 80 parts of sulphuric anhydride, $\frac{80}{233} = {\cdot}343$, so that

$0{\cdot}297 \times {\cdot}343 = {\cdot}1018$ SO_3 in 50 c.c. bisulphite $\times 2 = 0{\cdot}203$ per 100 c.c.

4. **Lime.**—Make a 10 per cent. solution of the bisulphite by taking 20 c.c. and making up to 200 c.c. with distilled water. Measure 20 c.c. of this 10 per cent. solution ($=2$ c.c. bisulphite) into a beaker, add about 150 c.c. distilled water and about 10 c.c. ammonic oxalate, about the same quantity of ammonic chloride, and about the same quantity of ammonic hydrate. Gently warm the liquid and allow to remain at rest for about 3 hours and then filter. (The filtrate is required for the following magnesia estimation.) Wash the precipitate with hot distilled water, dry, ignite, cool, recarbonate, heat gently, cool under desiccator, and weigh as calcic carbonate ($CaCO_3$).

Example:—

Weight of crucible + ash	6·588 grams.
Weight of crucible	6·500 „
	0·088

$0{\cdot}088 \times {\cdot}56 = 0{\cdot}049$ CaO in 2 c.c. bisulphite and $\times\ 50 = 2{\cdot}450$ lime (CaO) per 100 c.c.

5. **Magnesia.**—To the filtrate from the previously described lime estimation add about 10 c.c. ammonic or sodic phosphate and about a like amount of ammonic hydrate; stir well and set aside for 6 hours. Now filter, wash the precipitate with dilute ammonia, dry, ignite, cool, and weigh as pyrophosphate of magnesia $2(MgO)P_2O_5$.

Example:—

Weight of crucible + ash	6·511 grams.
Weight of crucible	6·503 „
	·008

The atomic weight of $2(MgO)P_2O_5$ is 222, and that of 2MgO 80, so that 222 parts of pyrophosphate correspond to 80 parts of magnesia, $\frac{80}{222} = {\cdot}36$. So that $0{\cdot}008 \times {\cdot}36 = {\cdot}00288$ magnesia in 2 c.c. bisulphite, which $\times\ 50 = 0{\cdot}150$ per 100 c.c.

6. **Chlorine.**—Take 25 c.c. of the bisulphite in a platinum or porcelain dish, evaporate to dryness over the water bath, moisten the residue with a little distilled water and again evaporate to dryness, repeating the moistening with distilled water and evaporation to dryness three times. Now dissolve the residue with hot distilled water and transfer to a filter paper and filter

collecting the filtrate in a clean porcelain dish. Wash the precipitate with hot distilled water and collect the filtrate in the same porcelain dish. Now add to the filtrate one or two drops of potassic chromate and titrate with a standard solution of argentic nitrate (p. 137) as in Water Analysis, p. 160.

Example.—25 c.c. of the bisulphite took 1·7 c.c. of argentic nitrate, so that as each c.c. of the silver solution corresponds to 0·001 gram of chlorine, $1{\cdot}7 \times {\cdot}001 = {\cdot}0017$ in 25 c.c. $\times\ 4 = {\cdot}0068$ gram Cl per 100 c.c.

To bring this to terms of common salt (sodic chloride) the atomic weight of chlorine (Cl) is 35·5, and that of sodic chloride (NaCl) 58·5.

Every 35·5 parts of chlorine therefore correspond to 58·5 parts of sodic chloride $\frac{58{\cdot}5}{35{\cdot}5} = 1{\cdot}645$, so we multiply by the factor 1·645 or, roughly, by $1{\cdot}65 = 0{\cdot}011$ NaCl per 100 c.c.

7. **Iron.**—Measure 20 c.c. of the bisulphite into a small beaker, add about 50 c.c. distilled water and a few drops hydrochloric and nitric acids and boil until the evolved steam no longer smells of nitrous or sulphurous fumes. Now cool, transfer to a 100 c.c. flask and dilute to mark (= 20 per cent. solution). Of this solution take 50 c.c. in a Nessler tube, add a drop or two of potassic ferrocyanide, and stand for a minute or two, when, if iron is present, a blue colour will have developed varying in intensity with the amount of iron. Now take a second Nessler tube, add 50 c.c. distilled water, a drop or two of potassic ferrocyanide, and run in from a burette a standard solution of iron (p. 137), until the colour, which develops on standing for a few minutes after each addition of iron, exactly matches in intensity that of the bisulphite solution.

Suppose the quantity of iron solution used equals 30 c.c.; then, as each c.c. of iron solution is equivalent to 0·1 milligram of iron $30 \times 0{\cdot}1 = {\cdot}30$ milligram of iron, and ${\cdot}30 \times 5 = 1{\cdot}50$ milligram iron in the 100 c.c. of 20 per cent. bisulphite solution. Therefore $1{\cdot}50 \times 5 = 7{\cdot}50$ milligrams per 100 c.c. of the bisulphite, or ·0075 gram.

It is usual, however, as in water analysis, to express the iron as ferric oxide (Fe_2O_3), so that as 160 parts of Fe_2O_3 correspond to 112 parts of iron $\frac{112}{160} = {\cdot}7$. We multiply ${\cdot}0075 \times {\cdot}7$ and obtain iron as ·0052 (Fe_2O_3) per 100 c.c.

8. **Hyposulphites.** — These bodies, if present, render the bisulphite utterly unfit for use. It is therefore only necessary

to detect their presence, no estimation being necessary; and, if found to exist, the bisulphite should be at once condemned as unfit for use.

Their detection is a simple matter; all that is needed is to add to the bisulphite concentrated hydrochloric or any strong mineral acid and raise to the boil, when, if hyposulphites exist, a yellow precipitate of sulphur is thrown down. The precipitate should not be confused with one of a white colour sometimes obtained by this treatment.

Combinations.—We have now to combine acids and bases, our determinations being :—

	Per cent.
Total sulphurous acid	6·974
,, sulphuric acid	·203
,, lime	2·450
,, magnesia	·150
,, chlorine	·006
,, iron	·005

We first take the ·203 sulphuric anhydride (SO_3) and saturate it with lime equivalent :—

80 parts SO_3 combine with 56 parts CaO.

Therefore $$\frac{\cdot 203 \times 56}{80} = \cdot 142 \text{ CaO.}$$

$(\cdot 142 + \cdot 203) = \cdot 345$ per cent. $CaSO_4$ (calcic sulphate).

·142 parts of lime having thus been disposed of, the remainder $(2 \cdot 450 - \cdot 142) = 2 \cdot 308$, so that this now requires to be combined with sulphurous acid (SO_2), the atomic weight of which is 64.

Therefore $$\frac{2 \cdot 308 \times 64}{56} = 2 \cdot 602 \ SO_2,$$

and $2 \cdot 602 + 2 \cdot 308 = 4 \cdot 91$ per cent. calcic sulphite ($CaSO_3$).

Having thus completely saturated the lime, we have now to deal with the magnesia and combine this also with sulphurous acid. The atomic weight of MgO is 40, so that $\frac{\cdot 15 \times 64}{40} = \cdot 24$ SO_2, combining with ·15 MgO to form ·39 per cent. magnesic sulphite ($MgSO_3$). The total amount of sulphurous acid is 6·974 per cent., and the amount required to saturate the lime (2·60) and magnesia (·24) added together = 2·84, which deducted from the total = 4·13 per cent. free sulphurous acid. The full analysis is therefore as follows :—

Analysis of Bisulphite of Lime expressed on 100 *parts by volume.*

Specific gravity . . .	1069·0
Total sulphurous acid . .	6·97 per cent.
Free sulphurous acid . .	4·13 ,,
Combined sulphurous acid .	2·84 ,,
Calcic sulphate . . .	0·34 ,,
Calcic sulphite . . .	4·91 ,,
Magnesic sulphite . .	0·39 ,,
Sodic chloride . . .	0·011 ,,
Ferric oxide . . .	0·005 ,,
Hyposulphites . . .	Nil.

The results of analysis are frequently required to be expressed on 100 parts by weight instead of on 100 parts by volume as above. In such instances, taking the present analysis as an example, the results would work out thus:—

The specific gravity of the bisulphite was found to be 1069; the actual weight of 100 c.c. is therefore 106·9 grams, or 100 parts by volume equal 106·9 parts by weight; therefore each figure of the analysis multiplied by $\frac{100}{106 \cdot 9}$ expresses the results in percentage on the latter.

Example :—

Total $SO_2 = 6 \cdot 97$ in 100 c.c.

Therefore $\frac{6 \cdot 97 \times 100}{106 \cdot 9} = 6 \cdot 52$ per cent. total SO_2 by weight.

Free $SO_2 = 4 \cdot 13$ in 100 c.c.,

$\frac{4 \cdot 13 \times 100}{106 \cdot 9} = 3 \cdot 86$, and so on with each item.

The results therefore stand :—

Analysis of Bisulphite of Lime expressed on 100 *parts by weight.*

100 parts weight . . .	106·9 grams.
Total sulphurous acid . .	6·52 ,,
Free sulphurous acid . .	3·86 ,,
Combined sulphurous acid .	2·65 ,,
Calcic sulphate . . .	0·30 ,,
Calcic sulphite . . .	4·59 ,,
Magnesic sulphite . . .	0·36 ,,
Sodic chloride . . .	0·11 ,,
Ferric oxide	0·004 ,,
Hyposulphites . . .	Nil.

Solid Sulphites.

Calcic Sulphite. Sodic Sulphite. Potassic Sulphite.

Calcic Sulphite.

The determinations to be made are:—

Lime.
Sulphurous acid.
Sulphuric acid.
Iron.

The same general methods are adopted as those given for bisulphite of lime, half a gram of the sulphite being dissolved in distilled water and made up to 100 c.c. at 60° F. (15·5° C.) and the analysis proceeded with as described.

In some instances it will be found that there is a proportion of the lime CaO or $CaCO_3$ uncombined with acid, the presence of which is indicated when the acids fail to satisfy the base; and, if desired, a determination of the alkalinity or carbonic acid may be made, as described under Water Analysis, to check such result.

Samples of *soluble or precipitated sulphite* contain an equivalent of water of crystallisation, the sulphite existing as $CaSO_3 + H_2O$; with such, the analytical results will of course not add up to 100 parts, even when the moisture has been determined, because the water of crystallisation is not expelled, except at higher temperatures than can be employed without decomposing the salt.

Sodic and Potassic Sulphite.

With these it is only necessary to determine the sulphurous and sulphuric acids and ferric oxide. The bases are not usually determined, but if required they can be done as described under Water Analysis.

Malt Analysis.

The following are the determinations to be made:—

1. Extraneous matters.
2. Defective corns.
3. Weight per bushel.
4. Steely corns and modification.
5. Specific gravity of 10 per cent. solution.
6. Extract per quarter.
7. Dry extract per cent.

8. Saccharification period.
9. Specific rotatory power of mash wort.
10. Acidity.
11. Moisture.
12. Matters soluble in cold water.
13. Ready-formed soluble carbohydrates.
14. Colour of wort.
15. Mineral matter or ash.
16. Total proteids or albuminoids.
17. Soluble proteids or albuminoids.
18. Insoluble proteids or albuminoids.
19. Diastatic power.

Sampling.—Care should be exercised in obtaining an average sample of the malt. With malt heaped up on the kiln or in store, a little should be taken from different parts of the bulk. With malt delivered in sacks, a sample from the top, middle, and bottom of several sacks should be selected. The well-known

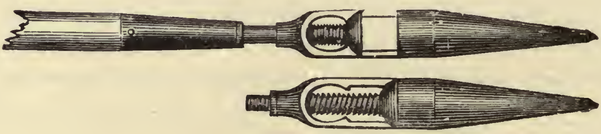


FIG. 65.

corn sampler (fig. 65) may be conveniently employed for this purpose. The various portions selected in this manner from any one particular class of malt should then be well mixed together and about 200 grams taken and screened.

1. **Extraneous Matters.**—A very simple and efficient screen for this purpose is the "Boby," which consists of a series of iron rods fixed together in the form of a frame which, enclosed in a box, may be given a fast or slow reciprocatory motion by hand at the manipulator's desire, or the well-known miniature screen shown in fig. 66. The refuse such as rootlets, tail corn, and other bodies falls through the mesh, from whence it may be collected and weighed. Thus, if we take a definite quantity of malt, screen it, and weigh the extraneous bodies, we naturally arrive at the quantity originally present in the sample. In one instance 200 grams were taken and screened; the screenings, together with seeds, stones, and other rubbish picked from the malt, weighed 2·4 grams = **1·2 per cent. extraneous matters.**

2. **Defective Corns.**—These include corns which are crushed, mouldy, half corns or extremely minute in size. Five hundred

corns are promiscuously separated from the sample and the percentage ascertained.

3. **Weight per Bushel.**—It is necessary to remember that malt just off the kiln is warm, and that when in this condition it is impossible to obtain its correct weight. It is wise in fact not to attempt the weighing of any malt until it has been off the kiln for at least 24 hours. The weight is ascertained by the instrument known as the "chondrometer" (fig. 67), which consists of a balance provided with a small measure to hold about a half-pint. The measure is filled with an average screened sample of the bulk from which the impurities such as stones, etc., have been removed; the surface is levelled with the "strike" and the weight ascertained. The weights bear the same proportion to the measure

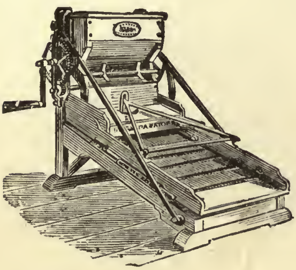


FIG. 66.

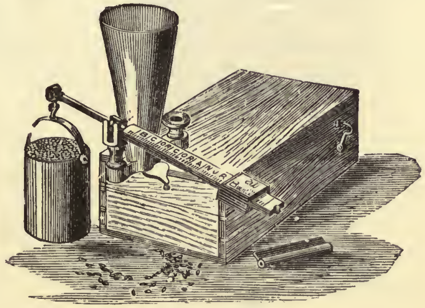


FIG. 67.

as pounds do to the bushel, so the weight in pounds per bushel is read off directly.[1]

4. **Steely Corns and Modification.**—Imperfectly vegetated or kiln-dried malts contain varying proportions of steely corns which are resistant to the bite and usually roughly detected in this manner. If selected and cut in two, it will be found that the starch has a peculiar cast and a vitreous or glassy appearance. It is advisable to estimate the steely corns, and this is most readily performed by means of the well-known "farinator" (fig. 68), which cuts through 50 or 100 corns with one stroke of the knife. Further, it has been found that light will pass through glassy but not through mealy corns. From this fact the instrument designated the "diaphanoscope" (fig. 69) has been constructed It consists of a box made of sheet metal, in the interior of which is placed a petroleum, gas, or electric lamp. The roof of the apparatus is provided with a number of slits, into each

[1] The results obtained must be regarded more as comparative values than reliable determinations of the actual bushel weight of the corn.

of which a corn of malt is placed. The quality of the malt is then judged from the relative transparency of the corns.

In examining malt by either the farinator or diaphanoscope, it is usual to operate on 500 corns. These are divided into three classes—floury, vitreous, and semi-vitreous; the numbers thus obtained, divided by five, giving the respective percentages of the different classes.

The old-fashioned "sinker" test was formerly much in vogue, and was considered capable of testing the percentage of semi-grown or dead corns in a sample of malt. It originated from a paragraph in Edward Lisle's work entitled *Observations in*

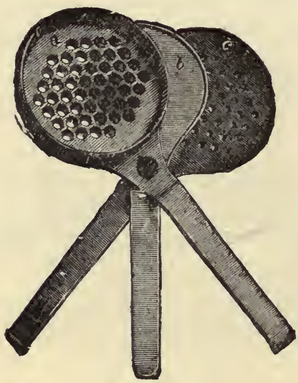


FIG. 68.

FIG. 69.

Husbandry, 1757, p. 205, where he states: "My maltster sent me malt which my butler was not pleased with. He said there were many grains in every handful of it which were not malted at all, and many grains that were but half-malted, of which I might be satisfied if I made trial in water; for the corns which were not malted at all would sink to the bottom, and the half-malted grains would swim on end like a fishing quill. I called for a basin of water to make the experiment and found it to be true."

The test is carried out by counting three or four separate hundred corns, or measuring off 300 or 400 corns in a small thimble or measure,[1] throwing the corns into a tumbler of water, well stirring for about half a minute, and counting those that sink and those that float perpendicularly.

The principle of the test depends upon the fact that corns

[1] This method is much quicker than counting, and the error in the number taken does not amount to more than a few corns, being inappreciable in the result.

which contain their starch, or a portion of it, in an unmodified, steely, or vitreous state, have a specific gravity greater than water, and sink when immersed. There are of course gradations in this difference of specific gravity, and many corns will be found to float in a perpendicular position which, on examination, may be found to have steely tips, but in which the balance of specific gravity between the friable portion of the corn and its steely part is so close as to prevent its subsidence. Also a shrivelled corn in which the plumule may have grown completely out, and yet not completed the whole of its modification, may occasionally be found floating. When this occurs it is in the case of a thin corn in which the growth of the plumule has apparently been too great to be sustained by the ordinary available food supply. A large portion of the cell walls, being in a steely state, has remained unmodified, and the embryo has used up the reserve starch along the dorsal side of the grain, depleting the endosperm cells. During the kilning process the corn becomes unduly shrivelled, and sometimes leaves sufficient air-space beneath the husk to turn the scale in favour of its floating.

Meacham, some two or three years ago, suggested the use of a 5 per cent. solution of methylated spirit in place of ordinary water; the decrease in specific gravity of the water thus produced bringing down those corns which, by reason of their being very slightly steely at the tips, otherwise float perpendicularly in ordinary water.

A further test is that of immersing the malt in *toluol*.[1] The specific gravity of malt immersed in this solution varies from 0·95 to 1·1; and, generally speaking, malts with specific gravity less than 1·0 are well modified, whilst those showing a specific gravity above this are not considered to be well modified.

On the whole, it may be stated that the sinker test with water or aqueous methylated spirit, and the method of judging modification from the specific gravity found by the aid of toluol, are decidedly unsatisfactory. The percentage of sinking corns varies so much from day to day, and is dependent upon so many conditions, that it is dangerous to condemn a sample of malt which may be very good from a chemical point of view and bad if done

[1] Toluol, or, as it is better known, toluene, is a hydrocarbon of the aromatic series, with a boiling point of 231·8° F. (111° C.) and a specific gravity 882·4 (water = 1000). It is used for taking the specific gravity of malt in precisely the same way as the specific gravity of a solid is usually taken with water, a method to be found in any elementary text-book on physics. It is necessary to use toluene instead of water when dealing with malt, because of the solvent action of the latter on certain constituents of the malt. Toluene, it is stated, has no solvent action on the malt.

by either of these methods. It is decidedly more advantageous and reliable to count or measure out several separate hundred corns and examine the degree of growth of the acrospire of each, so arriving at fair conclusions as to irregularity of growth or otherwise of the sample; whilst for steely, vitreous, or dead corns examination should be made by means of a penknife, separating out the dead or idle corns, vitreous corns, steely-backs, and steely-tips, classifying them in percentages.

Examination by means of the diaphanoscope or farinator is, however, reliable, so that we may resort to these methods of procedure and discard the sinker or specific-gravity tests as obsolete and next to useless.

The acrospire in the corns of good malt should be well and evenly grown, the average length being between two-thirds and three-quarters up the back. There should be few corns in which it either pierces the end or fails to extend to more than half up.

As the vitality of barley is the chief characteristic when judging its quality, so the growth of malt is also the main factor in considering its positive and comparative value.

The word growth has a wider significance than vitality; for, although no barley can possibly possess too much vitality, malt which is grown too much is of greatly reduced value. Any corn which remains unvegetated is not malt, but barley, and the paramount importance of the growth of the acrospire is consequently obvious.

To detect regularity in growth, a number of corns are taken promiscuously from a sample, say several lots of 200 each, and the number of corns which have grown to the various different lengths, or grown out, are determined by inspection; and the different groups of figures so obtained, divided by two, give the percentages. The following typical illustrations, giving percentages of growth, show the value of uniformity in samples from careful examination.

PERCENTAGE OF GROWTH.

	Good Malt.		Medium Malt.		Bad Malt.	
Acrospire grown out . .	. .	1	3	...	12	26
,, ,, $\frac{7}{8}$ up back .	9	4	7	4	19	21
,, ,, $\frac{3}{4}$,, .	64	80	64	79	42	28
,, ,, $\frac{1}{2}$,, .	26	9	14	11	17	9
,, ,, $\frac{3}{8}$,, .	1	3	6	...	...	1
,, started .	...	1	3	2	2	...
Dead corns	...	2	3	4	8	15
	100	100	100	100	100	100

Grinding.—Much controversy has taken place within recent years as to the degree of fineness to which the malt should be ground. Some prefer to grind to powder, others only to a coarse state such as is usual when grinding on a practical scale in the brewery. It naturally follows that two analysts, one grinding the malt to powder, the other only grinding so that the grist resembles the degree of fineness which is usual in practical operations, obtain very different results. In fact a difference of 3 or 4 lbs. per quarter results in the amount of extract obtained by grinding in both ways.

The old method of grinding, and at present largely practised, is by the use of the coffee-mill; and naturally, as the fluted cone is fixed or screwed up in these mills to different degrees, hardly two mills grind to the same degree. In any case, however, where such mills are employed, they should be arranged so that the interior may be cleaned before grinding.

In view of the fact that different analysts obtain different results by reason of the variance in the degree of fineness in grinding, it became obvious that a fixed standard was desirable, and some years ago it was agreed by many analysts to grind the malt to the finest powder. By this method, however, the extract obtained is far and away greater than can be obtained from the malt in practice; and, more particularly, steely corns which give little or no extract in the ordinary manner are, upon being reduced to powder, rendered amenable to the influences of heat and diastase.

In order to avoid these drawbacks, that is to say, enable the operator to grind to any desired degree of fineness and express the degree so that it may be known under what standard in this direction his analysis is conducted, Messrs Seck Bros., Ltd., of Dresden, Germany, some few years ago introduced their now well-known "Seck mill" for use either in the brewery or for laboratory purposes, the latter being shown in fig. 70 (p. 196).

In this mill the malt is crushed between two steel rollers as in actual practice. One of them is mounted in stationary bearings, whilst the other is capable of adjustment by means of an eccentric and lever, the adjustment being indicated on a scale graduated from 0 to 50.

The following figures represent the results obtained with the Seck mill by grinding to varying degrees, and they illustrate not only the wide difference in extract obtainable from one and the same malt, but also the fact already referred to, that in the

case of a steely malt these differences will be much greater than in that of a tender sample.

EXTRACTS PER 336 LBS.

	Ground to flour.	Seck 10.	Seck 20.	Seck 30.	Seck 40.	Seck 50.
English malt—tender	97·6	96·9	96·5	96·0	95·6	94·1
English malt—fairly tender	98·4	97·6	96·7	96·2	95·1	94·0
English malt—very steely	93·9	92·0	91·5	89·8	87·5	85·0
Californian malt .	91·6	90·6	89·6	89·0	86·6	85·2

For laboratory purposes the mill is, as a rule, set at 25°, this being the standard adopted on the Continent.

There is this, however, to be said with regard to the grinding of malt, that so far as the consulting analyst is concerned, with the Seck mill he should state in his analysis the degree he set the mill to before grinding. Whilst if a coffee-mill is employed, he should grind to powder, state this in his analysis, and by no means omit reference to the percentage of steely corns, since these, as before stated, with fine grinding give extract.

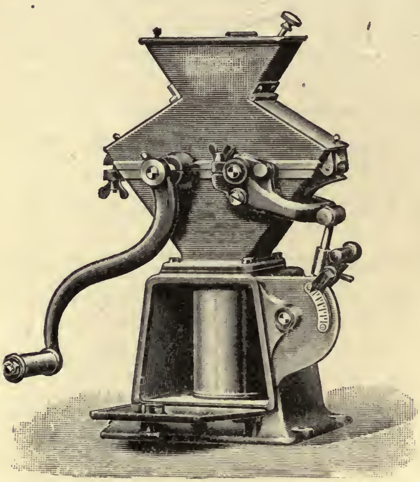


FIG. 70.

With the analyst on the brewery premises, if a Seck mill is employed it should always be set at the same degree; whilst in the absence of such mill a coffee-mill may be employed, since by its use the grain will always be ground to the same degree and the evaluation of his malts will always be judged from this and his comparisons uniformly drawn.

5. **Specific gravity of 10 per cent. solution.**
6. **Extract per quarter.**
7. **Dry extract per cent.**

The method which has been most widely adopted in this country for ascertaining the first and second of these estimations is that proposed by Heron[1] in 1888, and modified somewhat later,[2] which essentially consists as follows :—

Measure 400 c.c. of distilled water and run the same into a copper or enamelled beaker of about 1 litre capacity. Place the beaker containing a thermometer in a water bath in which the water is maintained by a thermostat (fig. 32, p. 27) at a constant temperature of 150° F. (65·5° C.). While the water is heating, weigh 50 grams of the screened malt in a small tared beaker; add three or four extra corns, grind in a coffee or Seck mill, transfer the grist to the same beaker and again weigh, withdrawing grist, if necessary, until exactly 50 grams are obtained.

When the 400 c.c. of water registers a temperature of 150° F. (65·5° C.), add the 50 grams of grist and stir well with the thermometer.

The miniature mash so made will shortly register an initial temperature of 150° F. (65·5° C.), and should be maintained at this temperature for 1 hour, which is more than ample time for complete saccharification to be effected.

8. **Saccharification Period.**—In order to record the time in which complete saccharification has taken place, a drop of the wort should be removed from the mash by the withdrawal of the thermometer. The drop should be allowed to fall from the thermometer upon a white surface—such as a white tile—then cooled, and a single drop of iodine solution added. The absence of a blue colour proves the absence of soluble starch, and hence saccharification, so far as it is capable of being accomplished by the infusion process, is complete, the modified starch of the malt having been transformed. The test for starch should be carried out 10 minutes after the mash has been made, and every 5 minutes afterwards, so long as a blue colour appears. It will be found, however, that the saccharification period of malts is usually from 20 to 30 minutes from the time the mash is made, so there is no perceptible loss of extract by the withdrawal from the mash of 4 or 5 drops.

Let us assume that the mash shows no blue colour with iodine after 25 minutes' infusion: the saccharification period is therefore 25 minutes. At the end of 1 hour's infusion the beaker containing the mash is removed from the water bath and the temperature of the contents raised to 160° F. (71° C.). The mash is then

[1] *Jnl. Soc. Chem. Ind.*, vii. 259.

[2] *Jnl. Fed. Inst. Brewing*, 1895, i. 116, and 1902, viii. 666.

transferred to a graduated glass jar through a copper or glass funnel, the beaker carefully washed out with distilled water, and the washings added to the graduated jar. The jar is then immersed in cold water so that the contents may cool, and the volume is finally made up to 515 c.c. at 60° F. (15·5° C.) with distilled water. The stopper is then inserted in the neck of the jar and the contents vigorously shaken and then thrown upon a filter and the filtered wort collected in a clean dry beaker. Every 100 c.c. of the filtrate will now contain the extract derived from 10 grams of malt. The specific gravity of the wort is now taken at 60° F. (15·5° C.) by means of the specific-gravity bottle (p. 33, fig. 36), and from this the extract is obtained by subtracting 1000 (weight of water) and multiplying the remainder by 3·36. This factor is arrived at as follows:—

W = weight in lb. of 1 quarter of malt.
V = volume of wort in c.c.
S = specific gravity of wort, less 1000.
p = quantity of malt in grams employed for the determination.
E = extract in lbs. per quarter.

The $$E = \frac{W\ V\ S}{1000\ p}$$

when $Y = 100$ and $p = 10$.

Then $$E = \frac{W\ 100\ S}{1000 \cdot 10} = \frac{W\ S}{100};$$

and if we assume that all malts weigh 336 lbs. per quarter, then

$$E = S\ 3 \cdot 36.$$

Example:—

1029·11 is found to be the **specific gravity.**

Then $29 \cdot 11 \times 3 \cdot 36 = 97 \cdot 80$ lbs. **extract per quarter.**

and $29 \cdot 11 \div 3 \cdot 86$[1] $= 7 \cdot 541$ dry solids per 100 c.c.

$7 \cdot 541 \times 10 = 75 \cdot 41$ lbs. **dry extract per cent.**

It will be noticed in the above experiment that, having taken 50 grams of grist and mashed with 400 c.c. of water, the bulk is finally made up to 515 c.c. Heron's contention is that the average volume of the grains from 50 grams of malt is 15 c.c. On this assumption, therefore, his wort would measure 500 c.c., and by the use of the factor 3·36 he obtains in the most direct manner the extract per 336 lbs.

In January 1891 a paper was published by Briant in the *Analyst* in which he takes exception to Heron's method for the

[1] Solution factor, p. 57.

determination of the extract of malt. Briant says: "This method is not, in my opinion, entirely satisfactory; Heron, it will be remembered, makes up to a constant bulk of 515 c.c., yet there is a very great variation in the amount of husk contained in malt samples. This is abundantly evident to anyone who is accustomed to the analysis of malts, and the amount of husk in a thin, foreign barley malt is manifestly very much larger than that in a thin-skinned, plump, bold English or Scotch barley malt. There is thus an error introduced due to this difference for which Heron has not made allowance."

Then he goes on to describe a method proposed by him which, he says, eliminates the error arising from the difference in amount of husk, and gives the true extract value of the malt sample.

Frew, in his paper on some notes on the analysis of malts,[1] a paper which, under a plea for simplicity and uniformity, appears to have been written with the set purpose of condemning in no measured terms the analytical English methods and extolling to the skies the German methods, says: "Heron's method of making up to a final volume of 515 c.c., based as it is upon an assumption, would be quite good enough if the volumes of the grains from all malts were equal, but this we know is not the case. The extract obtained by the use of this method from a heavy, thin-skinned, well-modified malt cannot be compared with that from a husky eastern malt of a somewhat steely character."

In answer to these criticisms, Heron [2] points out to what extent the difference in volume produced by the grains from different malts influence the extract. He says: "I have made extensive analyses of a good many malts and of various qualities in my time, and the smallest volume occupied by the grains, I found, was equal to 13 c.c., whilst the largest never exceeded 17 c.c.

"Now, suppose we had a malt the grains of which exactly occupied a volume equal to 15 c.c., and which gave an extract of, say, 90 lbs.; and another malt giving likewise an extract of 90 lbs., but whose grains occupied a volume equal to 17 c.c., but that the total volume of the wort was made up to 515 c.c.—what will be the error introduced? Why, the enormous amount of + 0·34 lb. On the other hand, with a malt whose grains occupy a volume of only 13 c.c. and giving also, say, an extract of 90 lbs., the error will be − 0·34 lb. But, as a matter of fact, such malts are very exceptional, and, in the great majority of cases, the error does not amount to more than + or − 0·1 to 0·15, and I think

[1] *Jnl. Fed. Inst. Brewing*, 8, 341.

[2] *Ibid.*. No. 6, 1902, 668.

such an error as this in the determination of the extract of malt may very well be neglected."

From this controversy the reader may take it for granted that Heron's method is to all intents and purposes as near perfection as anything can be, and is adopted in preference to others which introduce errors as great and in most cases greater than the infinitesimal amounts shown by him from an extensive number of experiments with different varieties and qualities of malt.

Determination of the "Full Theoretical" Extract.—The method of Heron's, already described, is employed to determine the extract of malts when mashed under conditions approximating to those in the brewery; but the whole of the possible extract is not obtained by such means, because even in the case of tender malts a small amount of the starch present is not hydrolysed, whilst with hard malts a large amount of starch often escapes conversion.

A method frequently employed to obtain the total available or "full theoretical" extract consists in treating the ground malt, previous to mashing, with a cold-water infusion of oats.

Ripe oats (not kiln-dried) contain a considerable amount of the enzyme cytase, and a cold-water infusion containing this enzyme is employed to act on the malt for the purpose of liberating the starch contained in the unmodified portions of the malt by dissolving the cell walls enclosing the starch granules.

Digest 50 grams of oats, rather finely ground, with 250 c.c. of cold water, for 3 or 4 hours, and filter. Mix 100 c.c. of the filtrate with 50 grams of the ground malt experimented with, and allow the mixture to stand at the room temperature for from 18 to 24 hours. Mash with 250 c.c. hot water, so as to obtain an initial heat of 151° F. (66° C.), and keep at this temperature for 1 hour. Transfer the mash to a 515 c.c. flask, and, after cooling, make up to the mark and filter, and proceed to determine the specific gravity of the wort in the usual manner. Before calculating the extract a correction must, however, be made for the specific gravity of the oat extract used in the experiment. Determine the specific gravity of the oat extract used, and subtract one-fifth of its excess gravity above 1000 from the specific gravity of the malt extract. The extract derived from the malt can then be calculated in the usual manner.

If the ordinary laboratory extract of the same malt has been determined, the difference between the two extracts is a measure of the modification of the malt. The so-called "coefficient of

modification" may be obtained by calculating the ordinary extract as a percentage on the "full theoretical" extract. For instance, if the ordinary extract of a malt is 87·6, and the "full theoretical" extract 95·7, then

$$\frac{87{\cdot}6 \times 100}{95{\cdot}7} = 91{\cdot}5,$$

the "coefficient of modification" of the malt, and the value is comparable with the "coefficient of modification" of any other malt found in a similar manner.

9. **Specific Rotatory Power of Mash Wort.**—Raise a small quantity of the 10 per cent. wort derived from the previously made miniature mash, by Heron's method, to the boiling-point, cool to 68° F. (20° C.), fill a 1 decimetre tube (100 mm.), place in the polarimeter, and take a reading. Let us assume the opticity in a 1 decimetre tube to be 9°.

In cases where the wort is too dark in colour to enable a correct polarimetric reading to be observed, such as when the malt mashed is highly dried or when colour malts are employed, it becomes necessary to decolorise the wort before polarising.

To do this take 50 c.c. of the boiled and cooled wort in a 100 c.c. flask, add a few drops of a 10 per cent. solution of lead acetate, make to mark with distilled water, agitate, and filter through a dry paper into a dry beaker. Fill a 1 decimetre tube with this solution at 68° F. (20° C.) and polarise.

Example:—

Opticity in 1 decimetre tube $= 4{\cdot}5°$.

$4{\cdot}5 \times 2 = 9°$ on 100 parts of wort, which on 7·54 dry extract

$$\text{in 100 parts of wort} = 9 \times \frac{100}{7{\cdot}54} = [a]_D\ 119{\cdot}4°.$$

10. **Acidity.**—This is determined by titrating 100 c.c. of the 10 per cent. wort at 60° F. (15·5° C.) with $\frac{N}{10}$ sodic hydrate, using litmus as indicator.

Example:—

100 c.c. wort required 1·5 c.c. $\frac{N}{10}$ alkali.

$1{\cdot}5 \times {\cdot}009 = {\cdot}0135 \times 10 = 0{\cdot}13$ per cent. on malt.

11. **Moisture.**—For the determination of the moisture percentage in malt, it is advisable to have at hand three or four small bottles of wide mouths and low build. The bottles should be numbered by marking with a file, and their weights carefully

ascertained and entered in a convenient book. Marked counterpoises are sometimes employed, such as are explained with the specific-gravity bottle.

In estimating the moisture percentage 5 grams of the malt are weighed, one or two extra corns added, and ground to a fine powder in a coffee or Seck mill. The powder is then transferred to the bottle and weighed, withdrawing grist, until exactly 5 grams are weighed. The bottle is then placed in the water-bath drying oven and left for 5 hours. This time should not be exceeded, as, if so, the sample gains in weight owing to oxidation. At the end of 5 hours the bottle is placed under the desiccator to cool, 1 hour being the usual time allowed for this purpose. The weight is now ascertained, the bottle again placed in the drying oven for a further hour, again cooled under the desiccator, and weighed.

Example:—

Weight of bottle + 5 grams grist .	105·513 grams.
Weight after drying	105·396 „
Moisture in 5 grams .	·117

$$\cdot 117 \times 20 = \mathbf{2{\cdot}34 \text{ per cent. moisture on malt.}}$$

Instead of weighing an exact 5 grams as per the foregoing experiment, any convenient quantity may be weighed, and a rule of three sum gives the result, thus:—

Weight of bottle	100·300 grams.
Weight of grist before drying . . .	5·370 „
	105·670
Weight of bottle + 5·370 grams grist after drying	105·544
	·126

$$\text{Then } \frac{\cdot 126 \times 100}{5{\cdot}370} = \mathbf{2{\cdot}34 \text{ per cent. moisture on malt.}}$$

The above-mentioned method of determining the moisture percentage in malts is at present considered sufficiently satisfactory for general purposes; but in view of the fact that slight oxidation of the constituents of the malt takes place during drying in this manner, a much more accurate though complicated method has been proposed, particulars of which may be found in a paper by Ford and Guthrie,[1] which consists in drying the sample (ex-

[1] *Jnl. Fed. Inst. Brewing*, No. 4, 1905, 326–332.

cluded from the ordinary air) in contact only with an atmosphere of carbonic acid gas.

Matters Soluble in Cold Water and Ready-formed Soluble Carbohydrates.

12. **Matters Soluble in Cold Water.**—The extract for the determination of matters soluble in cold water may be made so as to answer also for the determination of colour value, next described, and also, with dilution, for the estimation of diastatic power and soluble albuminoids. With this in view, instead of directly making a 10 per cent. solution as is usual, one of double strength may be conveniently prepared thus:—

Forty grams of the malt are weighed, ground to powder, and transferred to a stoppered bottle of about 1 litre capacity. 200 c c. of distilled water at 70° F. (21° C.) are next added, and the bottle set aside for 3 hours at a temperature of 70° F. (21° C.), shaking every half hour = 20 per cent. solution.[1]

At the end of the 3 hours the extract is filtered, and 50 c.c. at 60° F. (15·5° C.) are added to a 100 c.c. flask and boiled for 5 minutes. The contents are then cooled and made to mark at 60° F. (15·5° C.) = 10 per cent. solution.[2] This is then filtered, and the specific gravity taken, from which the matters soluble in cold water are determined by dividing the specific gravity, less 1000, by the factor 3·86.

Example:—

Specific gravity = 1006·20 − 1000 = 6·20

6·20 ÷ 3·86 = 1·606 dry solids per 100 c.c.

and 1·606 × 10 = **16·06 per cent. matters soluble in cold water expressed on the malt.**

[1] From this 20 per cent. solution the colour is determined as in experiment 14, and a 5 per cent. solution is prepared from it for the determination of diastatic power as in experiment 19.

Brown and Heron (*Jnl. Chem. Soc.*, 1879, 596) showed that the addition of sodium hydrate to the cold-water extract completely destroys diastatic action; and Ling, in view of this, proposes that the water, before being added to the bottle, should be rendered slightly alkaline by the addition of 0·02 per cent. caustic potash, or an equivalent amount of caustic soda or ammonia.

Morris has shown that there is no appreciable attacking and solution of starch unless the 3 hours' limit of standing is exceeded. The author found that whereas the matters soluble in cold water at the end of 3 hours were 15·36 per cent., at the end of 6 hours they were 20·33 per cent., and in 24 hours, 24·74 per cent.; so that time is a factor of great importance, and in no instance should the 3 hour limit be exceeded.

[2] From this 10 per cent. solution is also determined the ash as in experiment 15, and the soluble albuminoids as in experiment 17.

13. **Ready-formed Soluble Carbohydrates.**—From the matters soluble in cold water, which have been found to equal 16·06 per cent., the ready-formed soluble carbohydrates are estimated by deducting the soluble albuminoids, mineral matter, and acidity, thus:—

Matters soluble in cold water . .		16·06 per cent.
	Per cent.	
Soluble albuminoids . . .	2·66	
Mineral matter	1·08	
Acidity	0·13	
		3·87
		12·19

The ready-formed soluble carbohydrates are therefore 12·19 on 100 parts of malt, so that 10 grams malt contain 1·21 to correspond with 100 c.c. of wort.

Where the albuminoids, mineral matter, etc., are not determined, the ready-formed soluble carbohydrates are always approximately estimated by subtracting 4 from the matters soluble in cold water.

14. **Colour of Wort.**—In this determination it is necessary that the wort should possess a gravity near the Excise standard (1055° or 20 lbs.), or be calculated to the same. The 20 per cent. cold-water extract previously prepared for the determination of matters soluble in cold water answers this requirement, as it has been shown by Heron that as much colour is extracted from a malt by cold water as by water at 150° F (65·5° C.). The determination of colour is therefore most conveniently performed by filling a 1 inch cell of Lovibond's tintometer with the 20 per cent. cold-water extract, and employing the 52 series of yellow glasses.

Example.—A 20 per cent. cold-water extract, prepared as described, filtered and examined in the tintometer, using a 1 inch cell, required glass No. 5. **The colour value is therefore 5°.**

15. **Mineral Matter or Ash.**—This is determined by measuring 50 c.c. of the 10 per cent. cold-water extract at 60° F. (15·5° C.) and evaporating to dryness over the water bath in a tared platinum dish. The dish is then placed over a Bunsen flame and the residue cautiously ignited. At first the residue swells in the form of a semi-carbonaceous mass, but afterwards shrinks. Objectionable fumes are often given off, but may be obviated by applying a light. A strong flame may now be used, and the dish covered with a piece of platinum foil so as to

accelerate the oxidation of the carbonaceous matter.[1] The ignition should be continued until not a glowing particle can be seen in the ash when the latter is red-hot. Preferably, however, the dish is placed in a muffle, and the reduction to complete ash easily effected. When the ash is either grey or brown—the latter indicating the presence of iron—the dish is placed under the desiccator, and when cool, weighed. After weighing, the ash should be dissolved by means of a little hydrochloric acid, the dish carefully cleaned, ignited, cooled, and re-tared; a correction being necessary should the dish have lost weight, which is sometimes the case, especially if subjected to prolonged intense heat.

Example.—Fifty c.c. of the 10 per cent. cold-water extract, evaporated to dryness, incinerated to ash in a platinum dish, cooled, and weighed.

Weight of platinum dish + ash	16·394
Weight of platinum dish	16·340
	·054

·054 × 2 = **·108 per cent. ash on wort,**
and ·108 × 10 = **1·08 per cent. ash on malt.**

Proteids or Albuminoids.

There are several methods for estimating the proteids or albuminoids in malt, wort, sugar, beer, etc.; thus we have Kjeldahl's process,[2] which consists in digesting the substance in a flask for several hours with an excess of sulphuric acid at a temperature approaching the boiling-point of the acid, whereby the greater part of the organic nitrogen is converted into ammonia, and this conversion is completed by oxidation with potassic permanganate, which is added as a fine powder in very small quantities at a time. The addition of permanganate is continued until the solution becomes green, showing that an excess has been employed. When cold, the liquid is diluted with water, rendered alkaline with a strong solution of caustic soda, and then distilled into a receiver containing a measured quantity of standard acid (figs. 71 and 72). Kjeldahl's method

[1] It is usual to sulphate before incineration, the result being more quickly and reliably achieved. This is accomplished by adding 2 or 3 c.c. of sulphuric acid to the wort, evaporating, and burning off in the manner described.

[2] *Jnl. Chem. Soc.*, Abstracts, 1884, 364.

was quickly taken up by other investigators, C. Arnold[1] suggesting a slight modification and Gunning[2] proposing another.

We have also the older methods, such as burning the organic substance with copper oxide and copper so as to obtain all the nitrogen as a gas, and the well-known method by which the substance is mixed with soda lime and burned so as to decompose the nitrogenous substance to ammonia. Kjeldahl's process, however, either as originally proposed or with slight modification, is usually resorted to in ordinary routine work; but for a thorough investigation as to its accuracy, the reader is referred to the first volume of the *Transactions of the Guinness Research Laboratory.*

For all ordinary purposes, however, the following modification of Kjeldahl's method is sufficiently accurate:—

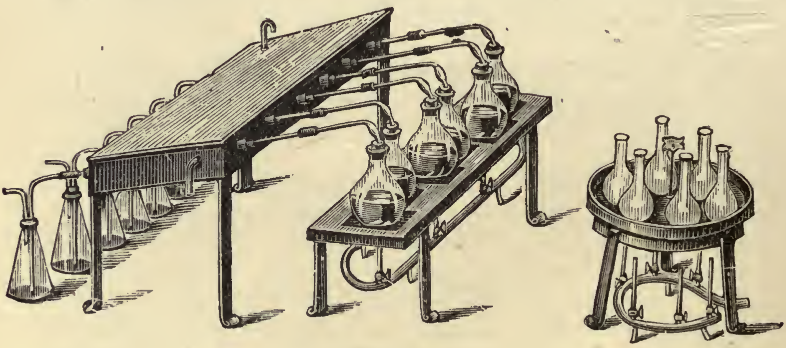


FIG. 71.

From 1 to 5 grams (according to richness in nitrogen) of the substance is introduced into a flask of hard Jena glass, and treated with 10 or 20 c.c. of strong sulphuric acid and 5 grams of potassic sulphate. The flask is then heated over a Bunsen flame very gradually, and after frothing has ceased, the heat is increased to brisk boiling and continued until the liquid gradually becomes clear and colourless. The contents of the flask are then cooled, washed into a distilling flask, a sufficient quantity of sodium hydrate to more than neutralise the acid is quickly added, and the flask then connected with the condensing apparatus and the ammonia distilled over into a flask containing a known quantity of a standard sulphuric acid. The loss of acidity is then finally determined in the usual way, and expressed as nitrogen or albumin. In figs. 71 and 72 we have a series of

[1] *Jnl. Chem. Soc.*, Abstracts, 1885, 930, and 1887, 78.
[2] *Ibid.*, 1889, 796.

flasks in which to digest the substance, a series of distilling flasks each connected with a condensing tube, and a series of flasks to contain the standard acid.

Example :—

16. **Total Albuminoids.**—Two grams of the malt are weighed, ground to powder, and placed in a boiling flask. Ten c.c. of strong sulphuric acid and 5 grams of potassic sulphate are added, and the flask placed over a flame and the contents gradually raised in temperature. After frothing has ceased, the heat is increased to boiling-point and the heating continued until the

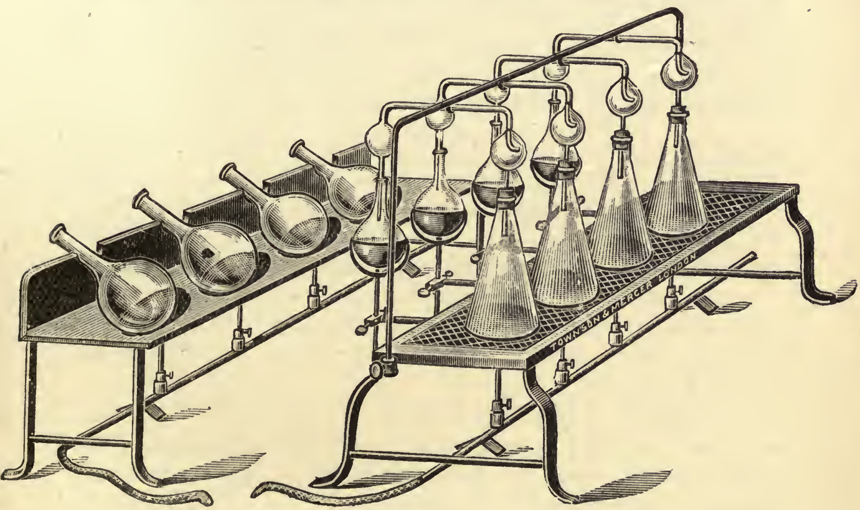


FIG. 72.

contents become colourless or possessing but a pale yellow tinge. The contents of the flask are now cooled, washed into a distilling flask with about 100 c.c. of ammonia free water, about 40 c.c. of sodium hydrate added, and a few pieces of pumice-stone or pipe-clay to prevent bumping. The flask is then connected with the distilling apparatus and the contents distilled, the distillate being collected in a flask containing 75 c.c. $\frac{N}{20}$ sulphuric acid. When about 100 c.c. of the distillate has been collected, the whole of the ammonia produced by decomposition of the proteid bodies will have passed over and have been absorbed and neutralised by the $\frac{N}{20}$ acid.

The acidity of the distillate is now tested by adding a few

drops of methyl-orange as indicator and titrating with $\frac{N}{20}$ ammonia; the number of c.c. required being deducted from the quantity of $\frac{N}{20}$ acid placed in the flask (75 c.c.), equals the number of c.c. of acid neutralised by the ammonia produced by the decomposition of the proteids originally in the malt.

Each c.c. of $\frac{N}{20}$ acid equals ·0007 nitrogen. Taking the albuminoids as containing 15·7 per cent. of nitrogen, we calculate the quantity of albuminoids to which the nitrogen corresponds. The factor 6·33 is used for this purpose. In titrating the distillate with $\frac{N}{20}$ ammonia, suppose the quantity required to neutralise the $\frac{N}{20}$ acid equalled 34·60 c.c. Then 75·00 – 34·60 c.c = 40·40 c.c. $\frac{N}{20}$ acid neutralised by the ammonia derived from the albuminoids originally contained in 2 grams of malt, and 40·40 × ·0007 = ·028280 × 50 = 1·414000 × 6·33 = **8·94 per cent. total albuminoids on the malt.**

A correction is necessary for the nitrogen in the reagents used and for the action on the glass apparatus, and it is advisable in all instances to perform a blank experiment and deduct the ammonia so found.

17. **Soluble Albuminoids.**—Ten c.c. of the 10 per cent. cold-water extract employed for the determination of matters soluble in cold water (p. 203) are run into a boiling flask and evaporated to dryness on the water bath. Ten c.c. of sulphuric acid and 5 grams of potassium sulphate are now added and the mixture heated, at first gently, until the first violent action is over, and afterwards strongly, until the liquid is decolorised. The liquid is now transferred to the distilling flask with about 100 c.c. of ammonia free water, about 40 c.c. of sodium hydrate added, and a few pieces of pumice-stone or pipe-clay to prevent bumping. The flask is then connected with the distilling apparatus and the contents distilled, the distillate being collected in a flask containing 50 c.c. $\frac{N}{20}$ sulphuric acid. When about 100 c.c. of the distillate has been collected, the whole of the ammonia produced by decomposition of the proteids will have passed over and have been absorbed and neutralised by the $\frac{N}{20}$ acid.

The acidity of the distillate is now tested by adding a few drops of methyl-orange as indicator and titrating with $\frac{N}{20}$ ammonia; the number of c.c. required being deducted from the quantity of $\frac{N}{20}$ acid placed in the flask (50 c.c.), equals the number of c.c. of acid neutralised by the ammonia produced by the decomposition of the proteids originally in the malt wort.

Each c.c. of $\frac{N}{20}$ acid equals ·0007 nitrogen. Taking the albuminoids as containing 15·7 per cent. of nitrogen, we calculate the quantity of albuminoids to which the nitrogen corresponds by the use of the factor 6·33, thus—

In titrating the distillate with $\frac{N}{20}$ ammonia, suppose the quantity required to neutralise the $\frac{N}{20}$ acid equals 44·0 c.c. Then 50·0 c.c. − 44·0 c.c. = 6·0 c.c. × ·0007 = ·00420 × 100 = ·42000 × 6·33 = **2·66 per cent. soluble albuminoids on the malt.**

18. **Insoluble Albuminoids.**—The *soluble* albuminoids deducted from the *total* albuminoids denote the *insoluble*, thus:—

Total albuminoids . . .	8·94 per cent.
Soluble ,, . . .	2·66 ,,
Insoluble ,, . . .	6·28 ,,

19. **Diastatic Power.**—A 5 per cent. solution of cold-water malt extract is prepared by taking 50 c.c. of the former 20 per cent. cold-water extract, detailed under the determination of matters soluble in cold water (p. 203), and making up to 200 c.c. with distilled water at 60° F. (15·5° C.).[1] Now take twelve

[1] It will be observed (p. 203) that the 20 per cent. cold-water extract is allowed to stand for exactly three hours at 70° F. (21° C.). The time of standing, up to six hours, does not interfere with the diastatic power, so long as the temperature is a constant 70° F. (21° C.), but if the temperature is increased the diastatic power of the extract varies, as the following will show:—

Diastatic power taken after cold-water extract stood—

	For 1 hour.	3 hours.	6 hours.
D. P.	29°	29·4°	29·4°

Temperature of digestion. °F.	°C.	Diastatic power.
70	21·0	29·4
78	25·5	37·7
95	35·0	57·1

Nessler or other glass tubes of about 50 c.c. capacity marked 1 to 12 (these are best arranged in order in the star apparatus devised by Reischauer), and add to each tube 10 c.c. of soluble starch solution prepared as described under Standard Solutions (p. 144). Now fill a graduated 5 c.c. pipette with the 5 per cent. cold-water extract, and add to the first tube ·1 c.c., to the second tube ·2 c.c., to the third tube ·3 c.c., and so on up to the twelfth tube 1·2 c.c. Shake the tubes and place them in the water bath, and maintain for *exactly* one hour at a temperature of 70° F. (21° C.). Now add to each tube 5 c.c. of Fehling's solution (*i.e.* 2·5 c.c. copper sulphate, and 2·5 c.c. alkaline tartrate), shake the tubes and place them in boiling water, and leave them for five minutes. At the end of this time remove the tubes and allow them to stand for five minutes. Now note the tube in which the blue colour has entirely disappeared. It often happens that there is a blue colour in one tube, and the next is overdone, that is, the colour is yellow. In such case the mean of the two is taken. For instance, suppose the third tube, containing ·3 c.c. of wort, is blue, and the fourth tube, containing ·4 c.c. of wort, is overdone. In such instance the mean, viz. ·35, is taken, and with practice it is easy to judge even more accurately, reading to one-fourth the value between each tube. Thus the third tube may be quite blue, the fourth only slightly overdone, in which case a reading of ·325 is taken.

Lintner, to whom we are indebted for this test (see p. 108), bases his standard as follows:—

The diastatic power of a malt is to be taken at 100 degrees, when ·1 c.c. of a 5 per cent. solution of malt extract just reduces 5 c.c. of Fehling's solution.

Example.—The end point was found to lie between the third and fourth tube; the amount of malt extract to be taken is therefore $\cdot 3 + \cdot 4 \div 2 = \cdot 35$, and $\frac{100}{\cdot 35} \times \cdot 1 = 28{\cdot}57°$.

This figure must be calculated upon water-free malt. Our malt is found to contain 2·34 per cent. of moisture; then $100 - 2{\cdot}34 = 97{\cdot}66$, and $\frac{100}{97{\cdot}66} \times 28{\cdot}57 = 29{\cdot}25°$.

The cold-water extract, however, itself contains a small amount of reducing sugars, and a correction for these must be made. This may be accomplished by conducting a blank experiment, or the more usual practice is to reckon that such reducing sugars are equal to 1·4°. We therefore

have $29{\cdot}25 - 1{\cdot}4 = 27{\cdot}85°$ diastatic power of malt on Lintner's scale.

Instead of performing the operation as just described, the following method may be adopted :—

Three c.c. of the 5 per cent. cold-water extract is allowed to act on 100 c.c. of a 2 per cent. solution of soluble starch at 70° F. (21° C.) for one hour in a 200 c.c. flask.

Ten c.c. of $\frac{N}{10}$ caustic alkali is then added, in order to stop further diastatic action, the liquid cooled to 60° F. (15·5° C.), made up to 200 c.c. with distilled water at the same temperature, well shaken, and titrated against 5 c.c. portions of Fehling's solution, using ferrous thiocyanate as indicator.

The titration is carried out as follows :—

Five c.c. of Fehling's solution are accurately measured into a 150 c.c. boiling flask. The converted starch solution is added from a burette, in small quantities at first of about 5 c.c., the mixture being kept rotated and boiled after each addition until reduction of the copper is complete, which is ascertained by rapidly withdrawing a drop of the liquid by a glass rod, and bringing it at once in contact with a drop of the indicator on a porcelain or opal glass slab.

Our analysis now works out as follows :—

Malt Analysis.

1.	Extraneous matters (per cent.)	1·2
2.	Defective corns (per cent.)	1·0
3.	Weight per bushel (lbs.)	42
4.	Steely corns (per cent.)	2
5.	Specific gravity (10 per cent. wort). Seck 25°	1029·11
6.	Extract per quarter (336 lbs.)	97·80
7.	Dry extract (per cent.)	75·41
8.	Saccharification period (minutes)	25·00
9.	Specific rotatory power of mash wort	$9° = [\alpha]_D 119{\cdot}4°$
10.	Acidity (per cent.)	0·13
11.	Moisture (per cent.)	2·34
12.	Matters soluble in cold water (per cent.)	16·06
13.	Ready-formed soluble carbohydrates (per cent.)	12·19

[1] In the examination of green malts, where the diastatic power often exceeds 100, the malt extract is diluted with an equal volume of water before being added to the tubes containing the soluble starch, the result being multiplied by 2.

14.	Colour of wort	5°
15.	Mineral matter or ash (per cent.) . .	1·08
16.	Total albuminoids (per cent.) . . .	8·94
17.	Soluble albuminoids (per cent.) . . .	2·66
18.	Insoluble albuminoids (per cent.) . .	6·28
19.	Diastatic power	27·85°

Wort Analysis.

Some few years ago the analysis of wort, as far as the carbohydrate constituents are concerned, consisted in first making a certain per cent. solution according to the gravity, taking a definite quantity, and conducting a Fehling's test, so as to obtain the cupric reducing power from which the percentage of maltose was first calculated, and afterwards the percentage of dextrose which such maltose would yield by hydrolysis. A further percentage solution of the wort was then made, and a definite portion inverted for a certain time at the boiling temperature, with the addition of 5 c.c. hydrochloric acid, after which it was cooled, neutralised, made to bulk, and the cupric reducing power again estimated and calculated to percentage on the wort. From these two results (before and after inversion) the former calculated dextrose was deducted from the reducing bodies obtained after inversion, and the result multiplied by ·9 gave the actual amount of dextrin. We thus obtained both the percentage of maltose and dextrin which the wort was supposed to contain. Now this was all very well in its way, but within recent years it has been shown that malt contains reducing sugars (ready-formed soluble carbohydrates) such as dextrose, levulose, and cane-sugar, so that unless we first take account of and deduct these we shall actually be attributing their presence in wort as due to the conversion of our starch, which is not so. Again, the inversion by the aid of acid results in the formation of inert bodies by reason of a decomposition of the end-products of inversion, the ready-formed cane-sugar being hydrolysed to dextrose, and some of the albuminoids transformed into actual cupric reducing bodies.

There can be no doubt under these circumstances that, with worts, the inversion method is to be condemned, and in its stead the carbohydrates estimated by the aid of the polarimeter. The total sugars are therefore now estimated from the cupric oxide reducing power, after deducting those ready formed, calculating to maltose, and then multiplying the maltose by the specific rotatory power of the wort, so obtaining the angle due to maltose,

and deducting this from the total rotatory power so as to obtain the angle due to dextrin; the latter angle is then divided by the rotatory power of 1 part dextrin, which gives at once the percentage of dextrin present in the wort.

Malt Wort.

The method proposed as regards malt wort was originally devised by Heron, and is essentially as hereafter detailed, the following being the determinations to be made:—

Hot mash wort	1. Dry extract per cent.
	2. Specific rotatory power.
	3. Cupric oxide reducing power.
	4. Acidity.
Cold-water extract	5. Matters soluble in cold water.
	6. Ready-formed soluble carbohydrates.
	7. Soluble proteids or albuminoids.
	8. Specific rotatory power.
	9. Cupric oxide reducing power.
	10. Mineral matter or ash.

In the ordinary course of events, had it been our primary intention to analyse the wort from the previously examined malt, most of these results would be carried forward. If they have not been determined, it becomes essential to make a 10 per cent. mash wort and carry out the experiments. We may, however, to save repetition, carry the results of our malt analysis forward, thus:—

1. **Dry Extract per cent. or Total Solids.**—Determined as explained under Malt Analysis.

Example.—The specific gravity of 10 per cent. hot mash wort $= 1029{\cdot}10 - 1000 = 29{\cdot}11 \div 3{\cdot}86 = 7{\cdot}54$ dry solids per 100 c.c. of wort.

2. **Specific Rotatory Power of Hot Mash Wort.**—Determined as explained under Malt Analysis.

Example.—Opticity of 50 per cent. solution at 68° F. (20° C.) in a 1-decimetre tube $= 4{\cdot}5^\circ \times 2 = 9^\circ$ on 100 parts of wort.

3. **Cupric Oxide Reducing Power of Hot Mash Wort.**—The determination of the reducing sugars is one of the most important estimations in brewing analysis, and as this depends largely on the reduction of Fehling's solution, it is advisable to give in some detail the exact method in which the reductions are made.

In estimating the cupric reducing power, careful attention must be paid to the following points :—

(1) The exact composition of the Fehling's solution as regards copper, and the nature and amount of the alkali it contains.

(2) The degree of dilution of the Fehling's solution.

(3) The restriction of the amount of copper reduced within certain prescribed limits.

(4) The mode of heating the solution, and the time occupied in reduction.

Having regard to the foregoing, the normal conditions under which all determinations should be made are :—

(*a*) The use of Fehling's solution prepared as described under Standard Solutions.

(*b*) The degree of dilution of the copper solution, taking into account the volume of the saccharine solution added, should be one part of Fehling's solution to one part of water.

(*c*) An amount of the reducing sugar should be taken which will give a weight of CuO lying within the limit of 0·15 to 0·35 gram.

(*d*) The diluted Fehling's solution should be heated in a suitable beaker in a boiling-water bath until the temperature is constant, and then the weighed or accurately measured volume of liquid containing the reducing sugar added, and the heating in the water-bath continued for exactly 12 minutes, the beaker being covered with a clock-glass during the whole period of heating. The filtration should be performed as rapidly as possible through a carefully folded paper, and the paper then dried and burned off in a tared crucible over a Bunsen flame in the usual way, and after cooling, the contents weighed as cupric oxide.

Example.—Fehling's solution is first diluted as follows :—

25 c.c. of the copper solution and 25 c.c. alkaline tartrate are added to a beaker together with 30 c.c. of distilled water. The beaker is then placed in the boiling-water bath, and when its contents are brought to the boiling-point 20 c.c. of the 10 per cent. mash wort are added, and the whole boiled for 12 minutes. The beaker is then removed and the contents filtered. A good filter paper should be used, or otherwise some of the CuO is liable to pass through. If such were the case, it would, after standing, be found at the bottom of the beaker which has received the filtrate, in which case it becomes essential to refilter. Wash the precipitate time after time, using for this purpose about 200 c.c. of boiling water, and finally wash with about 10 c.c. of strong

alcohol, then dry the filter in the water oven, transfer to a tared crucible and burn off, ignite to redness for 15 minutes, cool under the desiccator and weigh, repeating the heating, cooling, and weighing until the weight is constant.

Example :—

Weight of crucible and CuO . . .	6·780 grams
Weight of crucible	6·633 „
	·147[1]

·147 = amount in 20 c.c. of 10 per cent. solution of wort, or 2 c.c. of wort. Then $\cdot147 \times 50 = 7\cdot35$ grams in 100 c.c. of wort.

4. **Acidity.**—This is determined as in Malt Analysis by titrating 100 c.c. of the 10 per cent. wort with $\frac{N}{10}$ alkali, using litmus as indicator.

Example.—100 c.c. of 10 per cent. wort required 1·5 c.c. $\frac{N}{10}$ alkali. $1\cdot5 \times \cdot009 = \cdot0135$ acid in 100 c.c. of wort.

5. **Matters Soluble in Cold Water.**—These are determined exactly as in Malt Analysis.

Example.—Specific gravity of cold-water extract = 1006·20. $1006\cdot20 - 1000 = 6\cdot20 \div 3\cdot86 = 1\cdot606$ dry solids per 100 c.c. of solution.

6. **Ready-formed Soluble Carbohydrates.**—Also determined as in Malt Analysis.

	Per cent. on malt.	Per cent. on malt.
Matters soluble in cold water . .		16·06
Soluble albuminoids . . .	2·66	
Mineral matter or ash . . .	1·08	
Acidity	0·13	
		3·87
		12·19

The ready-formed soluble carbohydrates on 100 parts of malt = 12·19, so that 10 grams of malt contain 1·21 to correspond with 100 c.c. of wort.

7. **Soluble Proteids or Albuminoids.**—These are determined as in malt analysis, under Soluble Albuminoids.

10 c.c. of the 10 per cent. cold-water extract evaporated to dryness in a boiling flask ; 10 c.c. of strong sulphuric acid and 5

[1] This figure, it will be noticed, is rather low.

grams of potassic sulphate added, the contents warmed and then boiled until the solution is clear; then added to a distilling flask with about 100 c.c. distilled water, about 40 c.c. sodic hydrate added together with a few pieces of pumice, the flask connected with the distilling apparatus and the contents distilled, the distillate being collected in a flask containing 50 c.c. $\frac{N}{20}$ sulphuric acid.

100 c.c. of the distillate having been collected, the acidity is tested:—

Example.—50 c.c. $\frac{N}{20}$ acid required 44 c.c. $\frac{N}{20}$ alkali to effect neutralisation.

$50 - 44 = 6{\cdot}0$ c.c. $\times {\cdot}0007 = {\cdot}00420 \times 10 = {\cdot}04200 \times 6{\cdot}33 = {\cdot}26$ albuminoids in 100 c.c. of cold-water extract.

8. **Specific Rotatory Power of Cold-water Extract.**—A small quantity of the extract is boiled and then cooled to 68° F. (20° C.). A 2 decimetre tube is then filled with the same, placed in the polarimeter, and a reading taken. The reading, divided by 2, gives the reading of a 1-decimetre tube.

Example.—Opticity in 2-decimetre tube = 0·60°. 0·60 ÷ 2 = 0·30°.

9. **Cupric Oxide Reducing Power of Cold-water Extract.**—Determined as with the 10 per cent. hot mash wort (p. 213).

Example.—CuO obtained = ·282 amount in 20 c.c. ·282 × 5 = 1·41 gram on 100 c.c. of 10 per cent. malt extract to correspond with 100 parts of wort.

10. **Mineral Matter or Ash.**—Determined as per Malt Analysis. 50 c.c. of the 10 per cent. cold-water extract evaporated to dryness in a tared platinum dish, after sulphating; then ignited to ash, cooled, and weighed:—

Weight of platinum dish + ash	16·394
Weight of platinum dish	16·340
	·054

·054 × 2 = ·108 per cent. on wort.

We now deduct the figures obtained from experiments with our cold-water extract from the corresponding determinations of our hot wort, thereby arriving at figures due entirely to starch products. Our results are therefore worked out as follows:—

	Wort.		Cold-water Extract.		Conversion Products due to Starch.
Cupric oxide reducing power	7·35	–	1·41	=	5·94
Specific rotatory power .	9·00	–	0·30	=	8·70

Each gram of cupric oxide, according to Fehling's factor, equals ·7435 gram of maltose; we therefore obtain the amount of maltose in the wort by multiplying the cupric oxide obtained by this factor.

5·94 (CuO due to starch products) $\times$ ·7435 = 4·44 maltose in the wort expressed in percentage on the wort.

The specific rotatory power of 100 grams of maltose, when observed in a 1-decimetre tube, is $[\alpha]_{D\,3\cdot86} = 135\cdot9°$; therefore 1 gram = 1·359°. The amount of maltose calculated equals 4·44; so this, multiplied by 1·359, = 6·03 angle due to maltose. Deducting this angle from the total angle of starch products, we get the angle due to dextrin.

The specific rotatory power of 100 parts of dextrin = $[\alpha]_{D\,3\cdot86}$ 194·4°, or 1 part = 1·944; therefore the remaining specific rotatory angle, divided by 1·944, gives the amount of dextrin expressed in percentage on the wort.

Example.—Total angle = 8·70 − 6·03 = 2·67 due to dextrin, and $\frac{2\cdot67}{1\cdot944} = 1\cdot38$ dextrin in 100 parts of wort.

100 parts of the wort therefore contain:—

Maltose (due to starch conversion)	4·44
Dextrin (due to starch conversion)	1·38
Ready-formed soluble carbohydrates	1·21
Proteids or albuminoids	·26
Mineral matter or ash	·10
Other bodies by difference	·15
Total dry solids	7·54

It is usual to express the composition of wort in percentage on its dry extract, as this permits of a comparison of the composition irrespective of gravity. This is performed by ordinary rule of three, thus:—

Dry extract—

7·54 :	4·44 (maltose)	:: 100 :	58·87
„	1·38 (dextrin)	:: 100 :	18·32
„	1·21 (ready-formed sugars)	:: 100 :	16·05
„	·26 (albuminoids)	:: 100 :	3·44
„	·10 (ash)	:: 100 :	1·33
„	·15 (other bodies)	:: 100 :	1·99
			100·00

The specific rotatory power of the wort is similarly calculated on the dry extract:—

Dry Extract.		Specific Rotatory Power.	
7·54	:	9·0°	:: 100 : $[\alpha]_D 119·4°$.

Raw-grain Wort Analysis.

In our former analysis we have dealt with wort obtained entirely from malt, but it is hardly necessary to mention that similar analysis may be performed by conducting our mashing operation with a mixture of malt and *prepared* raw grain, such as flaked or granulated maize or rice, in such proportions as is carried on practically in any particular brewery. It may also be necessary to conduct our mashing operation with malt and *unprepared* raw grain, such as maize or rice grits. In such instances it therefore becomes necessary to take a similar proportion of the one or the other as is being used in practice, gelatinise the starch, and cool the same to 150° F. (65·5° C.) before mixing with our malt mash.

In all such instances we merely reduce the percentage of malt in accordance with the percentage of raw grain employed, obtaining the usual 10 per cent. wort both with our mash and our cold-water extract, our results being then obtained precisely as described in malt and malt-wort analysis. It is obvious, however, that this essential method of procedure with different percentages of raw grain, raises grave difficulties to the standardisation of analysis (see p. 231).

Mash-tun Wort Analysis.

In many breweries it is a general custom, and an excellent one, to test the wort obtained from every mash in order to ascertain whether the conversion has been rightly conducted and the proper ratio of dextrin to maltose obtained.

With pale ale and so-called "stock ale," the dextrin percentage should undoubtedly be greater than for a mild or running beer, so that by testing the mash-tun worts any change in character of materials used or variation in mashing or sparging may at once be detected.

Mash-tun wort to be tested should be collected from the taps at two different periods: first, a sample should be taken exactly 15 minutes from setting taps; and secondly, a further sample after the sparge liquor has got well through the goods, say

exactly 2 hours after setting taps. The two samples should then be well mixed together, boiled for a minute or two, cooled to 60° F. (15·5° C.), and the specific gravity taken. The specific gravity should then be reduced to 1050° and a polarimetric reading taken with the wort at a temperature of 68° F. (20° C.).

Example.—(*a*) Sample of wort collected from mash-tun taps 15 minutes after setting, sp. gr. 1078·96. (*b*) Sample taken 2 hours after setting taps, sp. gr. 1025·28.

The two samples mixed together = sp. gr. 1052·12. 50 c.c. of the wort at 1052·12, reduced in gravity to 1050 by the addition of 2·12 c.c. water, and the solution raised to the boiling-point.

Now, before a polarimetric reading can be accurately taken, the wort must be perfectly clear. Wort taken from the mash-tun taps, on cooling, is never clear; it therefore becomes necessary to render it so; and in order to do this, 100 c.c. of the wort at a specific gravity of 1050, after raising to the boiling point, is run into a 200 c.c. flask together with 10 c.c. of a 10 per cent. solution of lead acetate, made to 200 c.c. with distilled water, thoroughly mixed and filtered through a dry filter paper into a clean dry beaker = 50 per cent. solution.

The specific rotatory power is now taken in a 1 decimetre tube, with the wort at a temperature of 68° F. (20° C.); reading × 2 = opticity of the wort.

Copper-wort Analysis.

In the analysis of wort from the copper, the following are the determinations to be made:—

1. Specific gravity.
2. Dry extract per cent.
3. Cupric oxide reducing power.
4. Specific rotatory power.
5. Proteid bodies or albuminoids.
6. Mineral matter or ash.

From the third and fourth determinations we arrive at the percentage of dextrin and maltose in the wort.

As previously cited, malt contains ready-formed soluble carbohydrates; and unless we estimate their amount and deduct them from our results, we shall be attributing their presence to the conversion of starch at the mashing stage. It is therefore necessary to estimate the cupric oxide reducing power and the

specific rotatory power of a cold-water extract made from a sample of the malt employed in mashing, and deduct these results from the results obtained with the copper wort. We may then determine the albuminoids and mineral matter from the copper wort itself.

Now, provided any of these results have already been determined, such as they would have been had we already analysed the malt or a cold-water extract produced from it, our results may be carried forward; and it only becomes necessary to find the factor by which to multiply our cold-water extract results to make them correspond with a wort of the same percentage of dry solids as our copper wort.

The following example will make this clear:—

Sp. gr. of copper wort $= 1045 - 1000 = \frac{45 \cdot 0}{3 \cdot 86} = 11 \cdot 66$ dry solids per 100 c.c.

Sp. gr. of laboratory 10 per cent. wort $= 1029 \cdot 11 - 1000 = \frac{29 \cdot 11}{3 \cdot 86}$ $= 7 \cdot 54$ dry solids per 100 c.c.

$\frac{11 \cdot 66}{7 \cdot 54} = 1 \cdot 54$ factor by which to multiply 10 per cent. cold-water extract results in order to make them correspond with a wort of the same percentage of dry solids as our copper wort (viz. 11·66).

An analysis of our copper wort gives, say, CuO per 100 c.c. = 11·42.

An analysis of our copper wort gives, say, specific rotatory power = 14·43°.

Our cold-water extract results gave CuO per 100 c.c. = 1·41.

Our cold-water extract results gave specific rotatory power 0·30°.

Hence corrections to correspond with our copper wort are:—

CuO	$1 \cdot 41 \times 1 \cdot 54 = 2 \cdot 17$.
Specific rotatory angle . .	$0 \cdot 30^\circ \times 1 \cdot 54 = \cdot 46^\circ$.

Which give figures due to maltose and dextrin as follows:—

CuO	$11 \cdot 42 - 2 \cdot 17 = 9 \cdot 25$.
Specific rotatory angle . .	$14 \cdot 43^\circ - \cdot 46 = 13 \cdot 97^\circ$.

Then $9 \cdot 25 \times \cdot 7435 =$ **6·87 per cent. maltose on the wort,**
$6 \cdot 87 \times 1 \cdot 359 = 9 \cdot 34^\circ$ specific rotatory power due to maltose,
$13 \cdot 97^\circ - 9 \cdot 34^\circ = 4 \cdot 63^\circ$ specific rotatory power due to dextrin,
and $4 \cdot 63^\circ \div 1 \cdot 944 =$ **2·38 per cent. dextrin on the wort.**

The copper wort therefore contains :—

Carried forward. { Maltose	. .	6·87 per cent.
Dextrin	. .	2·38 ,,
Ready-formed soluble carbohydrates 1·21 × 1·54	.	1·86 ,,
Soluble albuminoids 0·26 × 1·54	. . . .	·40 ,,
Mineral matter 0·10 × 1·54	. . . . .	·15 ,,
		11·66

Which may be expressed as percentage composition thus :—

Dry extract—

11·66 : 6·87 : : 100 : 58·92 maltose.
,, 2·38 : : 100 : 20·41 dextrin.
,, 1·86 : : 100 : 15·95 ready formed soluble carbohydrates.
,, ·40 : : 100 : 3·43 albuminoids.
,, ·15 : : 100 : 1·29 mineral matter.

100·00

Should it be desired to express the maltose and dextrins in terms of malto-dextrins, this may be performed as hereafter described.

Malto-dextrins or Amyloins in Wort.

It will have been seen that in our former wort analysis we have returned the starch transformation products as maltose and dextrin only, but from a perusal of our remarks, p. 79, it is evident that the products of the hydrolysis of starch by diastase include not merely maltose and dextrin, but combinations of the two, termed malto-dextrins or amylöins.

Moritz and Morris[1] describe a method for the determination of the malto-dextrins based on the assumption that the free maltose is completely fermentable by primary yeast, any reducing sugars found after such fermentation being therefore due to combined maltose, existing as malto-dextrin; the combined dextrin being determined by hydrolysing it into maltose by means of cold-water extract, and then determining the increase in reducing sugars.

The method of analysis is as follows :—

(1) A diastatic solution to be used for hydrolysing the malto-dextrins is first prepared thus :—

200 grams of finely ground highly diastatic malt are added to

[1] *Text-book of the Science of Brewing.*

500 c.c. of distilled water, well stirred, and allowed to stand in a cool place over-night. It is then filtered, the bright filtrate being used for the subsequent determinations.

The reducing power of this solution is now ascertained:—

10 c.c. of the filtrate are diluted to 100 c.c., digested 1 hour at 130° F. (54·4° C.), then cooled, and the reducing power estimated on 10 c.c. (= 1 c.c. of cold-water extract).

$$CuO \text{ obtained} = 0{\cdot}074 \text{ gram.}$$

This correction must be made when the solution is employed.

(2) The percentage of dry solids in the wort is now determined by taking the specific gravity and dividing the excess weight over 1000 by the solution factor 3·86. Thus sp. gr. = 1045°. $\therefore \frac{45}{3{\cdot}86} = 11{\cdot}65$ per cent. dry solids.

(3) **Determination of Combined Maltose.** (*a*) *Reducing Power of Wort after removal of free maltose by fermentation with yeast.*—Fifty c.c. of the wort are taken, placed in a small flask, ·25 gram of washed pressed yeast added, the mouth of the flask loosely plugged with cotton-wool, the flask placed on the forcing tray, and the contents allowed to ferment at 80° F. (26·6° C.) for 48 hours.

The wort is now boiled to expel the alcohol, cooled to 60° F. (15·5° C.), diluted to 100 c.c., a small quantity of alumina or kieselguhr added to assist clarification, then filtered, and the reducing power determined on 25 c.c. of the bright filtrate.

$$CuO \text{ obtained} = 0{\cdot}196 \text{ gram.}$$

(*b*) *Reducing Power of the Wort after removal of free maltose and maltose in malto-dextrin, by diastatic hydrolysis and yeast fermentation.*—Fifty c.c. of the wort are fermented as previously described, but with the addition of ·25 c.c. of diastatic solution as well as yeast (to hydrolyse the malto-dextrins).

Fermentation being complete, the solution is boiled to expel the alcohol, cooled to 60° F. (15·5° C.), a little alumina or kieselguhr added, the whole made up to 100 c.c., filtered, and the reducing power determined on 25 c.c. of the bright filtrate.

$$CuO \text{ obtained} = 0{\cdot}130 \text{ gram.}$$

This is due to the reducing bodies in the wort, other than maltose, and must be deducted from the amount found in the former experiment 3 (*a*). Therefore ·196 − ·130 = ·066 CuO due to combined maltose. The 25 c.c. of reduced wort used contained

12·5 of original wort; therefore, by multiplying by 8, we have the CuO due to maltose per cent. on the wort.

$$\cdot066 \times 8 = \cdot528 \text{ CuO in 100 c.c. wort, and}$$
$$\cdot528 \times \cdot7435 = \cdot392 \text{ combined maltose.}$$

This, calculated on the dry solids, gives:—

$$\cdot392 \times \frac{100}{11\cdot65} = 3\cdot36 \textit{ per cent. maltose in malto-dextrin.}$$

4. **Determination of Combined Dextrin.**—(*a*) *Reducing Power of Wort.*—Two hundred c.c. of the wort are diluted to 100 c.c., 10 c.c. of this solution (= 2 c.c. wort) are now taken, and a gravimetric estimation of the sugar made, just as described under Wort Analysis.

CuO obtained = 0·282.

(*b*) *Reducing Power of the Wort after digestion with cold-water malt extract.*—Twenty c.c. of the wort are taken, 2·5 c.c. of the previously prepared diastatic solution added, and the whole maintained in a water bath for 1 hour at 130° F. (54·4° C.). The solution is now cooled and diluted to 100 c.c., and the reducing power determined in 10 c.c.

CuO obtained = 0·324.

As the cold-malt extract used contained some reducing bodies, the amount of CuO due to these must be deducted. As 1 c.c. of the cold extract gave 0·074 CuO, a deduction of 0·018 due to ·25 c.c. must be made from the total CuO obtained.

Total CuO	0·324
Deduction for cold-water extract . . .	0·018
	0·306

The CuO due to the maltose and reducing bodies naturally present in the wort amounted to 0·282 gram. Therefore 0·306 − 0·282 = 0·024 CuO due to maltose from the hydrolysis of the combined dextrin in 2 c.c. of wort, and 0·024 × 50 = 1·20 CuO in 100 c.c., and 1·20 × 706[1] = ·847 combined dextrin in 100 c.c. of wort, or 11·65 dry solids. Then $\cdot847 \times \frac{100}{11\cdot65} = 7\cdot27$ per cent. of combined dextrin on the dry solids.

[1] The factor ·706 is obtained by multiplying that for maltose, viz., ·7435 × ·95, as 1 part of maltose is produced from ·95 part of dextrin.

Our results expressed on the dry solids work out thus :—

Maltose in malto-dextrin	3·36
Dextrin in malto-dextrin	7·27
Total malto-dextrin . .	10·63

It will be seen that the method is rather lengthy and tedious, and is open to the objection that its accuracy must in a great measure depend upon the type of yeast employed to bring about the fermentation. It is a process, however, which gives some useful information and at times is necessary to carry out.

Raw-Grain Analysis.

Of this class of material we have unprepared varieties such as barley, sago, tapioca, oats, maize, and rice. Of these barley gives a wort possessing a raw, objectionable flavour, and therefore has not been found an advantageous material to employ; sago and tapioca are expensive, and oats are only of use for the production of a particular kind of stout, as, owing to their high content of albuminoids and oily matter, they produce definite flavour and a cloudy wort. We thus narrow down to maize and rice.

Maize contains from 3 to 5 per cent. of oil which suffers decomposition at the boiling temperature, imparting offensive odours to wort, and on this account cannot be employed in its raw state. Within recent years, however, means have been devised for removing the oil; and raw maize freed from oil, and now known as "grits," has become a valuable malt adjunct. The oil in maize is principally contained within the germ, so that it is merely necessary, in order to produce it practically free from oil, to grind it through fluted rollers and afterwards screen it with the help of an air-blast, by which the germ and hull and starchy portion may be separately collected. Rice as it comes into this country is dehusked, and is, practically speaking, free from oil and other deleterious substances, and hence is a unique brewing material.

We thus have raw maize grits on the one hand and raw rice on the other, either of which may be employed by the brewer. The cheapness of these raw materials in comparison to the large percentage of starch they contain, and the high extract derivable from their manipulation, is a strong factor in favour of their use, but unfortunately there exists the fact that it becomes necessary to first gelatinise the starch prior to mashing. In order to do this it is necessary, on the practical scale, to infuse the material

for about one hour at a temperature of 190° F. (87·7° C.) with constant stirring, and hence a specially constructed conversion vessel is generally used, so that the starch may be gelatinised and the solution cooled to a temperature below that at which diastase is destroyed before passing to the mash-tun or otherwise mixing in the ordinary malt grist. In order to obviate the necessity of the brewer carrying out this necessary prior starch gelatinisation, manufacturers years ago undertook the performance of this object, with the result that prepared maize and rice were placed on the market.

These materials are therefore now cooked so as to burst the starch cells, and are then dried, in which condition they may be directly employed in the mash-tun, the starch becoming more or less soluble at the ordinary initial mash temperature of 150° F. (65·5° C.).

The usual mode of preparation is to grind the material through fluted rollers (in the case of maize grits this will have already been done), and then pass it to a revolving perforated hopper which is jacketed so that steam may be injected and made to permeate the grain. Here the grain is maintained in contact with steam until gelatinisation of the starch is effected, after which it is passed to rollers which are internally heated by steam or gas. The rollers are so adjusted that one roll travels faster than the other, and in this manner the gelatinised starchy material as it passes through becomes flaked.

The flakes are finally passed to another perforated cylinder, enclosed with an iron casing, where they are kept in motion and subjected to a circulation of hot air until perfectly dry.

In the manufacture of granulated material the methods employed are similar, excepting that there is no necessity to pass through rollers, as when manufacturing flakes. It is not unusual to steep the material in an aqueous acid solution for some time and then wash it until the washings are neutral, before steaming.

In the analysis of raw grain it sometimes becomes necessary to estimate the percentage of starch, but so far as the brewer is concerned, it is more usual to determine the extract yield.

Prepared Raw Grain (Granulated and Flaked Maize and Rice).

The following are the determinations to be made :—

1. Oil.
2. Starch.
3. Extract per quarter.

15

4. Proteids or albuminoids.
5. Acidity.
6. Mineral matter or ash.
7. Moisture.

1. **Oil.**—This estimation is usually carried out by means of a Soxhlet fat-extracting apparatus (fig. 73), which consists of a water bath 16 cm. diameter, a constant water-level arrangement, a stand and burner, a metal ball condenser, a Soxhlet tube, and a distilling flask. The alcohol or ether, used for extracting the oil, is heated in the flask by means of the heated water in the bath, the vapour rising through the Soxhlet tube and being condensed falls back into the inner tube, where the material to be denuded of oil is placed, so extracting the oil. The condensed solvent slowly fills the extractor, rises to the level of the upper inner syphon, and then returns back again into the flask, the process being continuous.

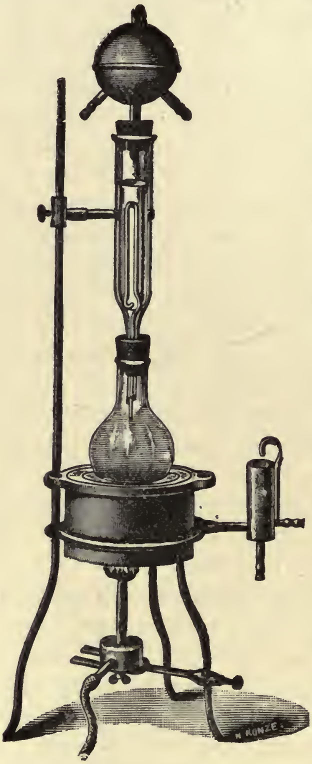


Fig. 73.

Care should be taken that there is no leakage in any of the connections or parts of the apparatus, as the solvent, being very inflammable, would immediately take fire should any of its vapour come in contact with a flame.

A loose plug of glass wool or cotton wool (free from fat) is inserted in the bottom of the Soxhlet tube, and on the top of this is placed the material to be experimented upon. Preferably, however, the material is placed in a thimble of bibulous paper (fig. 74) and the thimble with its contents inserted in the Soxhlet tube.

With raw grain, 5 grams, previously freed from moisture and ground to powder, are added to a thimble and placed in the Soxhlet. Forty c.c. of alcohol, specific gravity 0·920, are now added to the distilling flask and the whole Soxhlet apparatus

connected together. The distilling flask is now slowly heated by the water in the bath, and the alcohol then syphons off from the extractor; it will be found of the required specific gravity, viz. 0·900, whilst the temperature inside the extractor varies from 95°–104° F. (35°–40° C.). The extraction is carried on for 5 hours, after which the thimble is removed from the Soxhlet tube and its contents transferred to a beaker.[1]

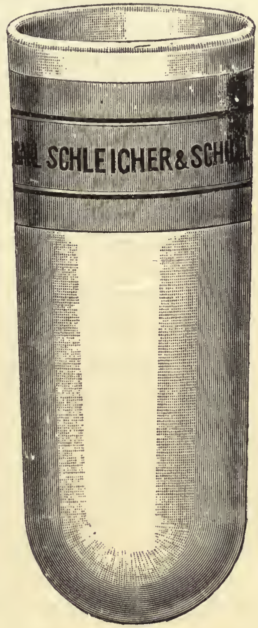


FIG. 74.

The distilling flask is now disconnected and the alcoholic solution is filtered through a filter paper into a tared beaker, both the flask and paper being washed with alcohol and the washings also collected. The beaker containing the filtrate is now placed on the water bath and the alcohol cautiously evaporated. The beaker with its residue is then placed in the drying oven for 1 hour, after which it is cooled under the desiccator and weighed, the drying, cooling, and weighing being repeated until the weight is constant.

The calculation is then as follows :—

Five grams of grain taken, the oil, after extracting, evaporating, drying, cooling, and weighing =

Weight of beaker + oil residue . .	17·282 grams.
Weight of beaker	17·120 ,,
	·162

$$\cdot 162 \times \frac{100}{5}\ \mathbf{3{\cdot}24\ per\ cent.\ of\ oil.}$$

2. **Starch.**—For the estimation of starch in cereals and other starch-containing vegetable substances, C. O'Sullivan's method[2] is, on the score of accuracy, by far the best which has been proposed. It consists in first denuding the grain of oil by lengthy, repeated decantation by the aid of solvents, then solubilising the starch and afterwards converting it into maltose and dextrin by

[1] The grain freed from oil is used in the next experiment for the estimation of starch.

[2] *Jnl. Chem. Soc. Trans.*, 1884, 1.

the aid of malt extract, calculating the starch from the conversion products obtained from both cupric oxide and polarimetric estimations. The method, however, is very tedious and requires a great deal of time; means had therefore to be taken to shorten the operation, and this has been accomplished [1] by extracting the soluble substances in a Soxhlet apparatus (as in our previous experiment) in the place of the ordinary process of washing by decantation, and after converting the starch calculating the same from the amount of maltose produced.

When starch is hydrolysed by active diastase under suitable conditions, there is a well-defined resting-stage in the reaction which closely approximates to the following equation:—

$$\underset{\text{Starch.}}{10C_{12}H_{20}O_{10}} + \underset{\text{Water.}}{8H_2O} = \underset{\text{Maltose.}}{8C_{12}H_{22}O_{11}} + \underset{\text{Dextrin.}}{C_{12}H_{20}O_{10}}.$$

Thus 100 parts of starch yield 84·4 per cent. of maltose.

Hence it follows that, if the starch of raw grain is so converted by diastase as to ensure its splitting up according to the above reaction, a mere determination of maltose from its cupric reducing power ought to give a direct measure of the original starch in the material without the necessity of polarimetric observation. The following method is therefore recommended:—

Five grams of the grain, freed from moisture and denuded of oil, as in our former experiment, are removed from the Soxhlet apparatus and transferred to a beaker; 100 c.c. of distilled water are added and the infusion gradually boiled for about 30 minutes, adding water, a little at a time, to replace that lost by evaporation. After cooling to 134·6° F. (57° C.), 10 c.c. of active malt extract [2] are added, and the conversion allowed to proceed for 1 hour. The solution is then boiled, filtered into a 200 c.c. flask, the residue well washed, and the volume made up to 200 c.c. at 60° F. (15·5° C.). The solution now contains the conversion products from the starch of 5 grams of the grain, and the precise amount of the maltose is ascertained from the cupric oxide reducing power as follows:—

Cupric Oxide Reducing Power.—This estimation is made as described in malt-wort analysis, 10 c.c. of the solution being added to 30 c.c. of Fehling's solution (15 c.c. copper sulphate

[1] *Trans. Guinness Research Laboratory*, 1903, 1, 79–91.

[2] The malt extract is prepared by weighing about 40 grams of malt, grinding the same to powder, and adding to a stoppered bottle together with 150 c.c. of distilled water. The infusion is set aside for about 1½ hours, occasionally shaking, and is then filtered bright.

and 15 c.c. alkaline tartrate) together with 50 c.c. of water, and the determination carried through as explained. CuO obtained = ·335 gram. The same estimation is made with 10 c.c. of the cold-water malt extract, so that correction may be made for the same. CuO obtained = ·152. Then ·335 – ·152 = ·183 × Fehling's factor ·7435 = ·1360 × 20 = 2·720 maltose in 200 c.c. or 5 grams of grain, which again multiplied by 20 gives 54·40 per cent. maltose.

The starch equivalent to this maltose is now estimated on the assumption that 84·4 parts of maltose correspond to 100 parts of starch; hence $\frac{54{\cdot}40 \times 100}{84{\cdot}4} = 64{\cdot}45$ per cent. **starch.**

3. **Extract per Quarter.**—The method of procedure in this estimation is somewhat similar to that employed for the estimation of starch, viz., it consists in saccharifying the starch of a known quantity of the raw grain by the aid of malt extract.

Twenty grams of the grain are weighed, placed in a beaker together with 120 c.c. of distilled water and 50 c.c. malt extract at a temperature of 150° F. (65·5° C.). The beaker is then placed on the water bath and the contents maintained at this temperature for 1 hour. At the end of this time the mash is transferred to a 200 c.c. flask, cooled, and made to mark at 60° F. (15·5° C.) with distilled water.

The mixture is then thoroughly shaken, filtered bright, and the specific gravity of the wort taken.

We have here the extract produced by the grain plus that due to the cold-water extract, and a correction must therefore be made for the latter. This is performed as follows:—

Of the original cold-water extract 50 c.c. are taken, slowly raised in temperature to 150° F. (65·5° C.), and maintained at this temperature with occasional stirring during 1 hour. The extract is then transferred to a 200 c.c. flask, cooled, and made up to bulk at 60° F. (15·5° C.) with distilled water. After thoroughly mixing, the solution is filtered bright and the specific gravity taken. We have here the extract due to the cold-water infusion. The gravity in our first experiment obtained from the mash, less that in our second experiment obtained from the cold-water extract, gives the gravity due to the grain. This gravity, multiplied by 3·32,[1] gives the extract yielded by 336 lbs. of the grain or extract per quarter.

[1] The grains obtained from 20 grams of granulated or flaked maize or rice are assumed to occupy a volume of 2·5 c.c. This being the case when the mash is made up to 200 c.c., the liquid portion will have a volume of

Formerly it was not unusual to determine the extract obtainable from prepared raw grain by mashing two-thirds of a malt of known extract and one-third of the grain, calculating the extract in the usual manner of the mixture of malt and raw grain. Obviously the extract of the malt had to be determined with the greatest accuracy, as any error would be multiplied in the calculation of the extract of the raw grain. To show what this error might amount to, Baker[1] determined the extracts of flakes by mashing in the first place equal quantities of maize and malt, and in the second place maize, together with two-thirds its weight of malt. Four experiments were conducted, in each case with the same maize, but with malts the extracts of which were known, but of different diastatic powers. The following table shows the results:—

Diastatic Power of Malt.	Extract per 336 lbs. of Maize when mashed with an equal Weight of Malt.	Extract per 336 lbs. of Maize when mashed with two-thirds its Weight of Malt.
	lbs.	lbs.
40	106·50	106·4
34	102·40	103·2
25	100·25	103·3
12	97·64	99·17

These results indicate the very marked influence which the diastatic power of the malt employed has on the yield of extract, and they also show that when more or less highly cured malts are used, the proportion of malt to maize in the *laboratory* mash is a matter of importance, if comparative results are to be obtained.

Where such a process has been followed, the manipulator will now entirely agree with Baker; but the method has long been superseded by the diastatic process devised by O'Sullivan, and already described, the accuracy of which has been recently confirmed,[2] the following table being given in proof thereof.

$200 - 2·5 = 197·5$ c.c. The factor to convert a 10 per cent. solution into lbs. per quarter is 3·36. As, however, the concentration of the liquid in the above experiment is less, the factor must be altered in proportion. The calculation is as follows:—

$$\frac{(200 - 2·5) \times 3·36}{200} = 3·318\text{; or, in round numbers, } 3·32.$$

[1] *The Brewers' Journal*, 1905, 41, 186.

[2] *Jnl. Fed. Inst. Brewing*, No. 5, vol. xi., 1905, 395, 398.

20 Grams of Flakes and 25 c.c. Diastatic Solution.

Diastatic Power of Malt from which the Diastatic Solution was obtained.	Extract per 336 lbs. of Flakes.
28°	102·2 lbs.
34°	102·2 ,,
65°	102·2 ,,

From these results it appears that the diastatic power of the malt used is of little importance when working with cold-water malt extract. The question arose, however, as to whether the full yield of extract was given when employing this proportion of diastatic solution. A second series of experiments was therefore conducted to ascertain what differences resulted from the use of varying amounts of cold-water extract. The following are the results :—

20 Grams of Flakes used.

Diastatic Power of Malt used.	Extract per 336 lbs. of Flakes.		
	25 c.c. Diastase Solution.	50 c.c. Diastase Solution.	75 c.c. Diastase Solution.
	lbs.	lbs.	lbs.
28°	102·3	104·0	104·2
65°	103·4	104·4	104·4

It will thus be seen that when using 50 c.c. of cold-water extract an increase over the amount yielded by 25 c.c. is shown, but that no further increase occurs when as much as 75 c.c. is used, and when using 50 c.c. it does not matter whether the malt has a diastatic power of as low as 28° or as high as 65°; hence 50 c.c. of cold-water malt extract is the proper quantity to employ for 20 grams of material.

It will be noted by these experiments that, although on the *laboratory scale* the details are such as to result in reliably obtaining the full extract capable of being yielded by the raw material, yet in practice the extract yield may fall short by as much as 7 lbs. per quarter, should the malt employed be of very low diastatic power.

Under these circumstances it is sometimes of as much importance to know what extract a definite proportion of raw material will yield in practice, when mashed in conjunction with a

certain class of malt, as it is to know what extract can be obtained analytically; hence the remarks on p. 218.

4. **Proteids or Albuminoids: Total Albuminoids.**—These are determined by Kjeldahl's method, as explained for total albuminoids under Malt Analysis (p. 207).

Soluble Albuminoids.—Twenty grams of the grain, ground to powder, are digested for $1\frac{1}{2}$ hours in 200 c.c. of distilled water at a temperature of 120° F. (48·8° C.), with occasional stirring. The infusion is then cooled to 60° F. (15·5° C.) and filtered = 10 per cent. solution. Fifty c.c. of the filtrate are now evaporated to dryness and the determination conducted as explained with soluble albuminoids in Malt-wort Analysis (p. 208).

Insoluble Albuminoids.—Difference between total and soluble albuminoids.

5. **Acidity.**—This is determined by titrating 50 c.c. of the 10 per cent. cold-water infusion as used for the estimation of soluble albuminoids with $\frac{N}{10}$ sodic hydrate, using litmus as indicator.

c.c. $\frac{N}{10}$ alkali used × ·009 × 10 = per cent. acidity on the grain.

6. **Mineral Matter or Ash.**—Determined from 50 c.c. of the 10 per cent. cold-water extract as employed for the determination of soluble albuminoids just described, and conducted as explained under Malt Analysis (p. 204).

7. **Moisture.**—Determined on 5 grams of the material, as explained under Malt Analysis (p. 201).

ANALYSIS OF PREPARED RAW GRAIN (GRANULATED AND FLAKED MAIZE AND RICE), CONDUCTED ACCORDING TO THE PRECEDING INSTRUCTIONS, GAVE THE FOLLOWING:—

	Granulated.		Flaked.	
	Maize.	Rice.	Maize.	Rice.
Oil	0·98	0·76	0·97	0·29
Extract per quarter (336 lbs.)	98·44	102·48	98·78	103·15
,, per cent.	75·90	79·01	76·16	79·53
Total proteids or albuminoids	9·20	8·74	9·50	8·53
Soluble ,, ,,	0·62	0·41	0·34	0 28
Insoluble ,, ,,	8·58	7·33	9·20	8·25
Mineral matter or ash	0·30	0·26	0·44	0·32
Moisture	10·72	7·83	6·30	7·43

Analysis of Unprepared Raw Grain (Raw Maize Grits and Raw Rice).

The determinations to be made are exactly the same as with the preceding prepared granulated and flaked maize or rice, and are carried out in precisely the same manner, excepting that in the determination of extract it becomes necessary to gelatinise and cool the starch before adding the malt extract.

Twenty grams of the ground grain are placed in a beaker together with 120 c.c. of distilled water and gradually boiled for about 30 minutes, adding water, a little at a time, to replace that lost by evaporation. The solution is then cooled to 150° F. (65·5° C.), 50 c.c. of malt extract added, and the operation carried on as described, from this point, with prepared raw grain (p. 229).

Analysis of Unprepared Raw Grain (Raw Maize Grits and Raw Rice), conducted according to the Preceding Instructions, gave the following:—

	Unprepared Raw Maize Grits.	Unprepared Raw Rice.
Starch	64·38	78·20
Oil	3·25	0·21
Extract per quarter (336 lbs.)	99·50	106·00
Total proteids or albuminoids	9·00	7·73
Soluble ,, ,,	0·50	0·33
Insoluble ,, ,,	8·50	7·40
Mineral matter or ash	2·20	1·54
Moisture	8·70	10·92
Cellulose, gums, etc., by difference	12·47	1·40

Analysis of Black, Brown, Amber, and Crystal Malts.

These materials are analysed in precisely the same manner as *prepared* raw grain (granulated or flaked maize or rice).

We have, however, to alter the factor by which to multiply the excess gravity between the mash and cold-water extract. The average bulk occupied by the grains from 20 grams of any of these malts is 6 c.c.; the factor to multiply the excess gravity between that of the malt and cold-water extract, calculated on the basis shown in the footnote, p. 203, is therefore 3·26. Hence in the determination of extract, specific gravity, minus 1000, multiplied by 3·26, gives lbs per quarter (336 lbs). There is one

other point, viz., with regard to black malt, it is often required to test its colour value. This is best performed by examining a ·01 per cent. boiled and cooled solution in a 1-inch cell of Lovibond's tintometer. One gram of the malt is weighed, ground to powder, and boiled in about 200 c.c. of distilled water for 15 minutes, afterwards cooled and made up to 1 litre = ·01 per cent. solution.

Analyses of Black, Brown, Amber, and Crystal Malts, conducted according to the Preceding Instructions, gave the following :—

	Black.	Brown.	Amber.	Crystal.
Extract per quarter (336 lbs.) . .	57·75	57·12	84·33	58·26
,, per cent.	44·30	44·04	65·02	45·07
Acidity of wort	·29	·23	·19	·17
Total proteids or albuminoids . .	6·11	7·13	7·62	8·71
Soluble ,, ,, . .	2·12	2·32	1·93	2·83
Insoluble ,, ,, . .	3·99	4·81	5·69	5·88
Mineral matter or ash . . .	·32	·29	1·20	·76
Moisture	5·37	6·23	4·14	2·12

Barley Analysis.

It is not often that the analyst is submitted samples of barley and asked to judge quality and give chemical analysis of the same, yet the importance of analytical results are now being realised.

The following are the determinations to be made :—

1. Extraneous matters.
2. Defective corns.
3. Weight per bushel.
4. Steely corns.
5. Character of endosperm.
6. Vegetative energy and capacity.
7. Oil.
8. Starch.
9. Proteids or albuminoids.
10. Mineral matter or ash.
11. Moisture.

1. **Extraneous Matters.**—Determined as with Malt (p. 190).

2. **Defective Corns.**—These include those corns which are broken, crushed, mouldy, damaged in dressing or by insects,

weathered in field, sprouted, heated in stack or very minute in size.

Five hundred corns are taken promiscuously from the sample and the percentages calculated.

3. **Weight per Bushel.**—As per Malt (p. 191).

4. **Steely Corns.**—As per Malt (p. 191).

5. **Character of Endosperm.**—Obviously, if barley will not germinate, vegetate, or grow, it is impossible to convert it into malt. Hence vitality of the germ is one of the most essential conditions of good barley, since should any sample possess more than an extremely small percentage of the grain incapable of germinating, there is considerable loss, as all ungerminated corns represent merely so much raw barley in the finished malt, and apart from yielding merely an infinitesimal extract at the mashing stage, form breeding-grounds for mould during vegetation on the malt-house floor, and result in the diastatic power of the malt being low.

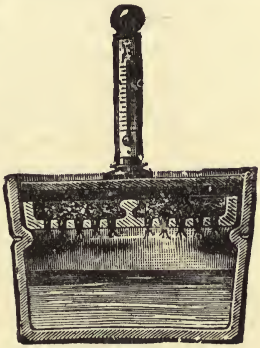


Fig. 75.

Defective vitality may arise from under- or over-ripeness, incipient germination in the ear owing to a wet harvest, death of the germ through heating in the stack, or improper storage, the attacks of insects and vermin, or damage to the grain during threshing.

The best test for vitality is to grow a sample of the barley; but this takes time, and the practical man, purchasing as he does in the open market, is therefore unable to avail himself of this method of procedure.

The test is usually carried out by taking 100 corns promiscuously from the sample without any picking or choosing, and placing them in an apparatus known as Coldew's germinator (fig. 75). It consists of a glass vessel with a constriction about $1\frac{1}{2}$ inches from the top, on which rests a porcelain plate provided with 100 perforations. Into each of these a corn of barley is inserted with the germ end downwards. The apparatus is nearly filled with water, the plate inserted, a small quantity of sand placed on top of the barley, and this is then moistened with water. A wooden cover with a layer of felt is then placed over the sand, and a small thermometer attached to the cover serves to indicate the temperature at which the experiment is being conducted. Upon

allowing germination to proceed for a few days, the relative degrees of vitality of the different corns can be readily estimated, and the number of sluggish and dead corns seen at a glance.

6. **Vegetative Energy and Capacity.**—According to the percentage of grain in a sample of barley which, when placed under favourable conditions for germination, vegetate within a definite time (this at the ordinary temperature being generally taken as 3 days), so is the **vegetative energy**; whilst the term **vegetative capacity** is employed to express the percentage number of corns which are found capable of germinating irrespective of time.

In a good sample of barley the vegetative energy should be not less than 90 per cent., and the capacity not below 95 per cent. The more closely these two characters correspond, the better, since any great variation plainly indicates that the sample would grow irregularly during malting.

7. **Oil.**—Determined as per Raw Grain (p. 226), 5 grams being taken and the extraction in a Soxhlet apparatus conducted for 5 hours.

8. **Starch.**—As per Raw Grain (p. 227).

9. **Proteids or Albuminoids.**—As per Malt (p. 205), 5 grams being taken, ground to powder, and treated according to Kjeldahl's method.

10. **Mineral Matter or Ash.**—As per Raw Grain (p. 232).

11. **Moisture.**—As per Malt (p. 201), 5 grams of the finely ground material being dried for 3 hours in the water oven.

Hop Analysis.

The following are the determinations to be made:—

1. Extraneous matters.
2. Soft and hard resins.
3. Tannin.
4. Moisture.
5. Sulphur.

1. **Extraneous Matters.**—A fair sample of the hops should be taken by opening the pocket or bale and taking a piece from the middle. This should include both the outside and interior of the hops. A flake should then be selected right across the hops so as to include as near as possible a portion of the outside and inside of the sample. The flake may be about 5 grams weight, and, without breaking it up, its exact weight should at once be ascertained. Say, for example, we select exactly 5 grams. This

sample is now placed on a piece of white glazed paper, and, by means of forceps, separated. Such a sample will be found to contain leaves, stalks, etc.; and these are then separated as accurately as possible and weighed, the amount so found being expressed as extraneous matters.

2. **Soft and Hard Resins.**—Having separated the extraneous bodies from the sample, as in the former experiment, and ascertained the weight of the same, the hops, which in this instance, including their moisture content, which must hereafter be reckoned with, weigh, say, exactly 4·5 grams. They are now transferred to a Soxhlet oil-extracting apparatus (fig. 73, p. 226);[1] 120 c.c. of petroleum ether is now added to the distilling flask, and the whole connected as shown in the figure. A light is now placed under the water bath and the temperature raised and maintained by the thermostat at a temperature of 155° F. (68·3° C.), which causes the ether to circulate continuously through the hops. The extraction, which proceeds slowly, is carried on for about 24 hours, the flask being then disconnected from the apparatus, its contents being filtered, whilst hot, through a small filter paper into a tared, wide-mouthed flask, and the filter paper being thoroughly washed with petroleum ether and the washings also collected.

The filtrate is now gently evaporated over the water bath, the soft resins being left as a residue. The flask is now placed on its side in the water oven, where it is maintained at a higher temperature than the boiling-point of ether, so that all traces of ether are got rid of. The flask is now placed under the desiccator and when cool weighed, the drying, cooling, and weighing being repeated until the weight is constant.

In the meantime the hops in the Soxhlet apparatus are further extracted by placing 120 c.c. of ordinary ether in the original flask, again connected with the apparatus, and the flask heated as before, excepting that the water in the bath is reduced in temperature to 135° F. (57·2° C.), and the circulation of ether carried on for a further 12 hours. The ethereal extract of the hard resins is thus obtained, filtered through the paper previously employed for the soft resins, washed with ether, and the evaporation and drying carried on as before. The analysis of both extracts is carried on in duplicate, and the mean of the results expressed in percentage on the dry hops, thus:—

Of the 4·5 grams of hops originally taken, we have to make a

[1] Instead of adding the hops direct to the Soxhlet tube, they are preferably placed in a thimble of bibulous paper (fig. 74, p. 227), this and its contents being placed in the Soxhlet apparatus.

correction for moisture, and as this is found (p. 242) to equal 9 per cent., the 4·5 grams contain 0·4, so that the actual hops used in our experiment equal 4·1 grams.

On extraction with petroleum ether the weight equalled 0·439 gram of soft resins, therefore $\cdot 439 \times \frac{100}{4 \cdot 1} = 10 \cdot 70$ **per cent. preservative soft resins.**

After extraction with sulphuric ether the resins obtained equalled 0·136 gram, so $\cdot 136 \times \frac{100}{4 \cdot 1} = 3 \cdot 03$ **per cent. non-preservative hard resins.**

Heron points out that it is exceedingly difficult to dry the residues from the ethereal extracts, and considers that a more reliable method for determining resins in hops is, instead of weighing the residues from the extracts, to weigh the hops from which the resins have been extracted. The author supports this opinion; but such method undoubtedly prolongs an already lengthy process, and gives results which are so infinitesimal as to hardly warrant its adoption.

3. **Tannin.**—The estimation of tannin in hops and various other astringent materials is not of the most satisfactory character. A great variety of methods have been proposed, the best of which appears to be the gelatine process as modified by Mulder, and the process by Löwenthal, modified by Neubauer.

The latter method is the one now generally employed, and has been worked out as satisfactorily as possible by Heron.[1]

It consists in titrating an aqueous hop extract with a weak standard solution of potassic permanganate, both before and after the removal of the tannin, by means of gelatine, using indigo solution as indicator.

Tannin is a very readily oxidisable substance, and its amount may be measured by the quantity of an oxidising agent, such as permanganate, taken to effect its oxidation. The addition of indigo is merely to act as an indicator, the indigo itself being readily oxidised into the so-called "white indigo." Its affinity for oxygen is, however, less than that of tannin, so that we are aware, when it has been decolorised, that all the tannin has been oxidised.

The indigo has then to be similarly titrated with permanganate and the addition of sulphuric acid; but without the aqueous hop extract, and upon determining the amount of permanganate which the indigo consumes, we deduct this from the total used in our first experiment.

[1] *Jnl. Fed. Inst. Brewing*, 1896, 165.

We have not yet, however, a direct indication of the amount of tannic acid present in the solution, because there are other bodies present which also have an affinity for oxygen, and therefore reduce permanganate; we have therefore to ascertain the extent of reduction effected by these bodies before we arrive at the real amount of tannin present. We cannot well precipitate or remove these bodies from the solution, but as an alternative we may effect the separation of the tannin, and, by noting the difference in reduction, deduce from this its amount. This we accomplish by the well-known power which gelatine possesses of precipitating tannin from its solution, and we proceed, according to Heron, as follows:—

Estimation of Tannin in Hops.

Ten grams of the hops are weighed out carefully and introduced into a wide-mouthed boiling flask graduated to 1005 c.c. and containing 900 c.c. of boiling distilled water.

The flask is then plunged into a bath of boiling water and digested under these conditions for 1 hour, shaking it occasionally during the digestion.

It is then taken out, cooled down to 60° F. (15·5° C.), and distilled water is added until the liquid in the flask reaches the containing mark; the flask and contents are then well shaken, and the extract so obtained is filtered as bright as possible.

As a rule it is difficult to get the infusion to filter bright, but the addition of a little kaolin facilitates the brightening of the filtrate to a marked degree.

One hundred c.c. of the filtrate represent the extract obtained from 1 gram of hops; the liquid is made up to 1005 c.c. instead of 1000 c.c., because it is found, as the mean of several experiments, that the volume occupied by 10 grams of hops, when completely exhausted, equals 5 c.c.; or, the 10 grams of hops may be weighed out in a tared beaker, digested in a water bath of boiling water with 1000 c.c. of distilled water for 1 hour, cooled down to 60° F. (15·5° C.) and then weighed, adding distilled water, if necessary, until the weight equals 1010 grams plus the tare of the beaker.

By this digestive process a complete extraction of the tannin is made in 1 hour.

It is advisable to see that in making the extract the water is actually boiling, for by working in this way the results are more uniform.

The extract of hops having been obtained, the next point is the determination of the tannin contained therein.

In order to perform this, the three standard solutions, viz., potassic permanganate (p. 144), indigo, and gelatine, detailed respectively on p. 145, are required, as well as a solution of sulphuric acid (50 grams concentrated sulphuric acid diluted with distilled water to 1 litre).

Fifty c.c. of the hop infusion are introduced into a shallow white porcelain dish of about 1 litre capacity, 20 c.c. indigo solution added, and 500 c.c. of good water; the permanganate solution is then added from a graduated burette in a rapid succession of drops, the liquid in the basin being kept vigorously stirred all the time with a glass rod: towards the end of the reaction the blue colour produced by the indigo changes to a light yellowish-green, rapidly changing into a golden yellow.

The titration is finished when this yellow colour is produced. When the distinctly blue colour begins to disappear, the permanganate must be run in more slowly and added very cautiously. Two such determinations should be made, and the mean of them taken, the number of c.c. of permanganate required for the 20 c.c. of indigo solution subtracted, and the remainder, multiplied by 2, represents the amount of total oxidisable bodies in 100 c.c. of the hop extract. Let this be denoted by a.

In order to determine the amount of permanganate required by the indigo solution, 20 c.c. of indigo solution should be added to 500 c.c. of water, and then the permanganate run in as already described.

One hundred c.c. of hop infusion are next taken and carefully poured into a wide-mouthed, 16 oz. flask, 100 c.c. of gelatine solution are added, the mixture in the flask well shaken, then 50 c.c. of the dilute sulphuric acid added, the flask shaken as before, then a teaspoonful of kaolin added, and the contents of the flask thoroughly well shaken once more, after which filtration may at once be proceeded with.

One hundred c.c. of the filtrate are introduced into the porcelain dish along with 20 c.c. of indigo solution and 50 c.c. of water, and the titration with permanganate proceeded with as before. The mean of two experiments, less the amount of permanganate required for the indigo, multiplied by 2·5, represents the amount of oxidisable bodies in 100 c.c. of the extract after the tannin has been removed; denote this by b. Then $a - b$ will equal the number of c.c. of permanganate required to oxidise the tannin in 100 c.c. of the extract.

Now, if we know the exact amount of hop tannin which 1 c.c. of permanganate is equal to, it will be very easy to calculate

the percentage of tannin present in the hops. It is true, Hayduck states, that 1 c.c. of permanganate is equivalent to, or oxidises, 0·002026 gram tannin, presumably hop tannin; but as this is a very much higher number than the one given by Neubauer for oak tannin several years ago, Heron does not care to adopt it without further confirmation.

Under these circumstances he considers the best plan is to express the number of cubic centimetres of permanganate required to oxidise the hop tannin in terms of oxalic acid, a standard which is easily and exactly verified; and certainly, for general purposes, that is, for the determination of tannin in substances other than hops, it is imperative that such a standard should be used. It is well known now that the tannins derived from different sources differ very much in many of their properties, and give different values when acted on with potassium permanganate; so that not until these different tannins have been isolated in a state of purity, and their values determined in terms of permanganate, can we determine with any degree of confidence or accuracy the exact amount of tannin in the substance under examination. Now we *can* measure exactly the amount of permanganate by means of oxalic acid, so that all our results by this method are strictly comparable for each individual tannin-containing substance. If therefore c be the quantity of permanganate required to oxidise 10 c.c. of $\frac{N}{10}$ oxalic acid, and 10 grams of hops have been employed in making 1000 c.c. of extract $\frac{a - b \times 6{\cdot}3}{c} = x$, where x is the percentage of tannin expressed in terms of pure oxalic acid.

Sometimes the gelatine solution has a very slight reducing action itself on the permanganate, so that it is advisable to make a blank experiment with each freshly made-up lot of gelatine solution; and where it is found that it possesses any reducing action, due correction for this must be made.

It is also a good plan, at starting a titration, to use the first experiment as a standard colour test for the succeeding ones.

The following example will serve to illustrate the working of the process:—

50 c.c. infusion 20 ,, indigo solution	} required .	30·9 c.c. permanganate.
20 ,, ,, ,,	alone required	20·25 ,, ,,
		10·65

Total oxidisable bodies—

100 c.c. in hop extract, required $10{\cdot}65 \times 2 = 21{\cdot}30$ c.c. $= a$.

After precipitation with gelatine—

100 c.c. filtrate, 20 ,, indigo solution	required .	24·7	c.c. permanganate.
20 ,, ,, ,,	alone required	20·25	,, ,,
		4·45	
Correction for gelatine .	.	1·95	
		2·50	

Oxidisable non-tannin bodies in 100 c.c. hop extract $= 2{\cdot}5$ c.c. $\times 2{\cdot}5 = 6{\cdot}25$ c.c. $= b$.

Hence

$$a = 21{\cdot}30$$
$$b = 6{\cdot}25$$
$$c = 31{\cdot}6,$$

and according to the formula

$$21{\cdot}30 - 6{\cdot}25 = 15{\cdot}05,$$

and $\dfrac{15{\cdot}05 \times 6{\cdot}3}{31{\cdot}6} = 3{\cdot}00$ per cent. tannin expressed in **terms of oxalic acid.**

4. **Moisture.**—This is determined by drying a convenient weight of the hops in the water oven, or preferably over sulphuric acid *in vacuo*, for 3 hours or longer, until the weight is constant. The main proportion, if not all, of the oil is driven off by this process; but as it does not exceed 0·5 per cent., the error this involves is not great, whilst if necessary a correction for this may be made.

Example.—5·211 grams of hops dried in the water oven for 3 hours, cooled under the desiccator, and weighed.

Loss in weight $= 0{\cdot}470$ gram.

$$\frac{0{\cdot}470 \times 100}{5{\cdot}211} = 9 \textbf{ per cent. moisture.}$$

5. **Sulphur.**—Sulphur may be readily detected in hops by generating hydrogen in contact with them and examining the escaping gas for sulphuretted hydrogen, very small quantities of which may be detected either by its offensive smell of rotten eggs, or by the production of a black coloration when allowed to impinge upon paper moistened with a solution of lead acetate, or come in contact with a solution of the same.

A convenient method of conducting this test is to generate hydrogen from zinc and dilute sulphuric acid in a small bottle containing about 200 c.c. of water and fitted with a cork, through which passes a bent tube to conduct the gas from the generating bottle into a small beaker containing a little lead acetate solution. (The Tyrer-Marsh apparatus, fig. 78, p. 274, is best employed for the generation of the gas.) After the gas has been allowed to pass for a short time, if the materials are pure, no change will take place in the lead solution. This being proved, the cork is removed from the bottle and a few hops, together with a little potash lye, are added, the cork being immediately replaced. If the hops contain sulphur, the nascent hydrogen will combine with it, producing sulphuretted hydrogen (SH_2) which passes into the lead solution, revealing its presence by the production of a black precipitate of lead sulphide, thus:—

$$Zn + H_2SO_4 = ZnSO_4 + H_2.$$
$$H_2 + S = SH_2.$$

Provided sulphur is detected as above described, the experiment is repeated, using 10 or more grams of hops and passing the sulphuretted hydrogen fumes into a definite quantity of lead acetate. The precipitated lead sulphide is then filtered, washed, dried, ignited, cooled and weighed, the sulphur being calculated from the weight so found. One part of lead sulphide (PbS) = 0·1345 sulphur.

An analysis conducted according to the foregoing instructions gave the following:—

MID-KENT HOPS.

Preservative soft resins	10·70 per cent.
Non-preservative hard resins	3·03 ,,
Tannin	3·00 ,,
Moisture	9·00 ,,
Sulphur	Nil.

BEER ANALYSIS.

The following are the determinations to be made:—

1. Original gravity.
2. Present gravity.
3. Dry extract.
4. Alcohol.
5. Acidity.

6. Specific rotatory power.
7. Colour.
8. Proteids or albuminoids.
9. Mineral matter or ash.
10. Iron.
11. Salicylic acid.
12. Malto-dextrins, etc.—
    - Fermented matter.
    - Low-type malto-dextrins.
    - Combined maltose.
    - Combined dextrin.
    - Free dextrin.
    - Unfermentable reducing residue.
13. Brilliancy.

1. **Original Gravity.**—The specific gravity of a wort, as determined by the brewer by the aid of a saccharometer, when about to enter the collection upon which the duty is to be charged, that is to say, before fermentation has commenced, is known as the original gravity. In course of time the wort ferments, the yeast organism splits up or decomposes sugar or fermentable bodies which results in the formation, as chief products, of alcohol and carbonic acid, the former remaining in the liquid (excepting a small proportion which is carried off mechanically with the carbonic acid gas which is largely evolved) and from its low specific gravity diminishing the specific gravity of the liquid. The original specific gravity of the wort is thus lowered during fermentation; but according to the lowering of the specific gravity, so are other bodies in fairly definite proportion formed. From every 100 parts of sugar decomposed, 52 parts of alcohol and 48 parts of carbonic acid, with traces of other bodies such as succinic acid, glycerin, etc., are produced; hence if we remove the alcohol from a definite quantity of beer, and make up the beer to the same bulk as before with distilled water, we shall be able to ascertain the specific gravity of the liquid minus alcohol; or, in other words, of the matter still remaining as unfermented extract in the beer; and by ascertaining the specific gravity of the separated alcohol, we may find how much fermented extract it represents, since, as before stated, every 100 parts of the extract have produced fairly definite quantities of other substances.

From these two factors we therefore arrive at the original gravity. There are several methods for determining the original gravity

of beer, but the "distillation process" is the best and most reliable, and is the one adopted in the analytical department of the Board of Inland Revenue. The various methods have been carefully investigated by Graham, Hoffmann, and Redwood, and from their observations the following table has been drawn up and adopted.

SPIRIT INDICATION, WITH CORRESPONDING DEGREE OF GRAVITY LOST IN MALT WORTS BY THE "DISTILLATION PROCESS."

Degree of Spirit Indication.	·0	·1	·2	·3	·4	·5	·6	·7	·8	·9
0	0·0	·3	·6	·9	1·2	1·5	1·8	2·1	2·4	2·7
1	3·0	3·3	3·7	4·1	4·4	4·8	5·1	5·5	5·9	6·2
2	6·6	7·0	7·4	7·8	8·2	8·6	9·0	9·4	9·8	10·2
3	10·7	11·1	11·5	12·0	12·4	12·9	13·3	13·8	14·2	14 7
4	15·1	15·5	16·0	16·4	16·8	17·3	17·7	18·2	18·6	19·1
5	19·5	19·9	20·4	20·9	21·3	21·8	22·2	22·7	23·1	23·6
6	24·1	24·6	25·0	25·5	26·0	26·4	26·9	27·4	27·8	28·3
7	28·8	29·2	29·7	30·2	30·7	31·2	31·7	32·2	32·7	33·2
8	33·7	34·3	34·8	35·4	35·9	36·5	37·0	37 5	38·0	38·6
9	39·1	39·7	40·2	40·7	41·2	41·7	42·2	42·7	43·2	43·7
10	44·2	44·7	45·1	45·6	46·0	46·5	47·0	47·5	48·0	48·5
11	49·0	49·6	50·1	50·6	51·2	51·7	52·2	52·7	53·3	53·8
12	54·3	54·9	55·4	55·9	56·4	56·9	57·4	57·9	58·4	58·9
13	59·4	60·0	60·5	61·1	61·6	62·2	62·7	63·3	63·8	64·3
14	64·8	65·4	65·9	66·5	67·1	67·6	68·2	68·7	69·3	69·9
15	70·5	71·1	71·7	72·3	72·9	73·5	74·1	74·7	75·3	75·9
16	76·5									

The details of the distillation process are as follows:—

A 200 c.c. flask is filled to the mark with the beer at a temperature of 60° F. (15·5° C.). If the beer is flat, this is easily accomplished; if, however, the beer is in much condition it is necessary to filter it through a dry filter paper into a dry beaker in order to expel the gas and measure the beer with accuracy. The expulsion of the gas is advantageous also from the fact that during distillation frothing is not likely to take place as it otherwise would. Instead of filtering the beer, the same object may be brought about by pouring it from one beaker to another.

Having accurately measured 200 c.c. of the beer at 60° F. (15·5 C.), it is transferred to a capacious distilling flask, the 200 c.c. flask is then washed out with a little distilled water, and the washings added to the beer; the flask containing the beer is then

connected to the still apparatus (fig. 33 or 34, pp. 29–30), a flame is placed beneath, the condensing water turned on, and distillation commenced.

The distillate is collected in the 200 c.c. flask originally used in measuring the beer, and the distillation continued until about two-thirds of the liquid has passed over, when the process is stopped and the flask removed.

The spirit has now been all expelled from the beer in the boiling flask, and is contained together with water in the distillate, whilst the liquid left in the boiling flask is now known as the residue.

To estimate the amount and value of the spirit, the contents of the 200 c.c. flask are now made up with distilled water to the original bulk (200 c.c.) at 60° F. (15·5 C.), thoroughly agitated, and the specific gravity taken by means of the specific gravity bottle.

The actual specific gravity, compared with the weight of water (1000), is then deducted from that number, and this result is termed the "spirit indication." From this indication the amount of decomposed or fermented extract is calculated. Thus if the specific gravity of the spirit has been found to be 991·5,[1] then the spirit indication would be—

$$1000 - 991{\cdot}5 = 8{\cdot}5.$$

By referring to the table previously shown, we find that 8·5 = 36·5 degrees of gravity lost during fermentation.

We have now to ascertain the value of the extract still remaining unattenuated in the beer, viz., the "residue." In order to do this the residue in the boiling flask is transferred to the 200 c.c. flask, the boiling flask repeatedly rinsed out with distilled water, and the washings added to the 200 c.c. flask. The liquid is then cooled and finally made up at 60° F. (15·5° C.) to 200 c.c. with distilled water and the specific gravity taken as with the spirit. Assuming this is found to be 1024·0, the original gravity of the beer would be—

Degrees fermented	36·5
Degrees unfermented . .	1024·0
	1060·5

degrees, which expressed in lbs. per barrel = 60·5 × ·36 = 21·78 lbs. per barrel.

[1] The present gravity (1015·5), minus this figure (991·5), should equal the gravity of residue (1024·0), which in this case it does.

In determining the original gravity of beers containing over 0·10 per cent. of acidity, a correction must be made as described under Acidity.

It should also be remembered that the original gravity of a beer, as determined by analysis and calculated by table, is always below that of the unfermented wort, the difference usually amounting to about 2 degrees.

2. **Present Gravity.**—The present gravity of beer should always be determined when testing the original gravity, since it shows to what extent the beer has attentuated, and serves as a check upon the result, thus:—

Let us assume that by the specific-gravity bottle we find the specific gravity of the beer to be 1015·3.

Now, as we have already determined the specific gravity of the alcohol, we have merely to deduct the "spirit indication" from the weight of the residue, to obtain the present gravity of the beer. Thus:—

Specific gravity of residue already found . .	1024·0
Spirit indication	8·5
Present gravity . . .	1015·5

while a direct experiment with the specific-gravity bottle gave 1015·3, thus corroborating the results within 0·2 degree, which is generally the difference between calculated and observed present gravities.

Original Gravity as determined by the Alcoholmeter.—To obviate the tedious and laborious methods of determining the original gravity of beer by the evaporation or distillation methods, an instrument was devised years ago by Field, and named by him the "alcoholmeter." This instrument has lately been improved upon by Manley, and gives fairly accurate results. The previously mentioned distillation process, however, should be carried out in all cases where extreme accuracy is desired; although it must be remembered that the original gravity determination is of little use by itself, it being necessary to test the carbohydrate constituents in a beer before a general opinion of its character can be expressed.

The principle of Field's or Manley's alcoholmeter consists in the fact that all spirituous liquors boil at a lower temperature than water, so that the larger the quantity of alcohol in any beer the lower will be its boiling-point. Hence by determining the boiling-point of water—which, it must be recollected, varies a

degree or two daily according to barometric pressure—and afterwards determining the boiling-point of the beer under examination, the carbohydrate substances, which must have been present in the wort and have been hydrolysed to alcohol, are estimated and the original gravity of the beer determined.

To save any complicated tables or calculations the alcoholmeter is fitted with a scale showing specific gravity degrees 0 to 95, divided into tenths for the purpose hereafter explained.

The instrument, a sketch of which is shown (fig. 76), consists of a small boiler (*c*) into which is poured about 120 c.c. of water or beer, and boiled by a small spirit-lamp placed underneath. The boiler is closed by placing on the cap which carries a mercurial glass tube the bulb of which dips into the liquid in the boiler, the remainder of the tube being continued, and by the side of which is arranged the scale (B) showing specific gravity degrees. The scale is rendered movable by the turning of the screw shown at the top of the instrument. By the side of the mercurial tube and scale is a small cylinder (A) with an open tube passing through it and through the lid.

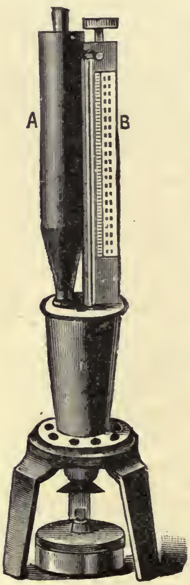


Fig. 76.

About the same quantity of cold water is added to this cylinder (which acts as the condenser) as beer or other liquid placed in the boiler, so that when a spirituous liquor is being boiled, the evolved alcohol is condensed and drops back into the boiler through the open tube.

In conducting a specific gravity determination we proceed as follows:—

Example.—About 120 c.c. of cold water is placed in the boiler, the same quantity added to the top cylinder, the lamp lighted and the water boiled. The mercury rises to almost the top of the tube, and when constant the scale is adjusted by the top screw so as to fix the boiling-point of water for some hours at least during the day. The water is then emptied out of both the boiler and the condensing cylinder, about 120 c.c. beer added to the boiler and the same quantity of cold water to the cylinder, and the boiling continued. The mercury then rises to a certain height, according to the quantity of alcohol present in the beer, and according to the height so is the degree read off from the

graduated scale. Say, for example, the scale having been set to 0 (zero) from the boiling-point of water, the mercury now rises to 55 from the boiling of the beer; the specific gravity of the beer as due to the alcoholmeter is therefore 55, which, added to the present gravity of the beer, determined by a small saccharometer supplied with the instrument, shows at once the original gravity. Thus alcoholmeter 55 and present gravity 1010 = 1065° original gravity.

3. **Dry Extract**.—This is ascertained from the specific gravity of the unfermented extract or residue obtained when determining the original gravity. It is useful in the sense that from it the constituents in the unfermented extract may be expressed on the dry solids, thus establishing a means of comparison between the solid matter in beers of different gravities and degrees of attenuation.

It may be determined by evaporating a definite bulk of beer to dryness in a tared platinum dish till the weight is constant and then calculating into terms of 100 c.c. During the evaporation, however, several of the constituents of the beer are oxidised, and the results are by no means satisfactory (see Solution Factor, p. 57), hence for all ordinary purposes it is more usual to employ the solution factor 3·86; thus—

Specific gravity of residue was found to be .	1024·00
Weight of water	1000·00
	24·00

$24{\cdot}00 \div 3{\cdot}86 = 6{\cdot}21$ per cent. **dry extract**.

4. **Alcohol**.—From the specific gravity of the spirit the per cent. of absolute alcohol by weight and the per cent. of proof spirit is ascertained by reference to the following table:—

[TABLE.

ALCOHOL TABLE.

Specific Gravity at 60° F.	Absolute Alcohol per cent. by weight.	Per cent. of Proof Spirit.	Specific Gravity at 60° F.	Absolute Alcohol per cent. by weight.	Per cent. of Proof Spirit.	Specific Gravity at 60° F.	Absolute Alcohol per cent. by weight.	Per cent. of Proof Spirit.
995·0	2·74	6·02	992·8	4·02	8·81	990·6	5·39	11·79
994·9	2·79	6·13	992·7	4·08	8·94	990·5	5·45	11·92
994·8	2·85	6·26	992·6	4·14	9·07	990·4	5·51	12·05
994·7	2·91	6·39	992·5	4·20	9·20	990·3	5·58	12·20
994·6	2·97	6·52	992·4	4·27	9·36	990·2	5·64	12·33
994·5	3·02	6·63	992·3	4·33	9·49	990·1	5·70	12·46
994·4	3·08	6·76	992·2	4·39	9·62	990·0	5·77	12·61
994·3	3·14	6·89	992·1	4·45	9·75	989·9	5·83	12·74
994·2	3·20	7·02	992·0	4·51	9·88	989·8	5·88	12·87
994·1	3·26	7·16	991·9	4·57	10·01	989·7	5·96	13·02
994·0	3·32	7·29	991·8	4·64	10·16	989·6	6·02	13·15
993·9	3·37	7·40	991·7	4·70	10·29	989·5	6·09	13·30
993·8	3·43	7·53	991·6	4·76	10·42	989·4	6·15	13·43
993·7	3·49	7·66	991·5	4·82	10·55	989·3	6·22	13·59
993·6	3·55	7·79	991·4	4·88	10·68	989·2	6·29	13·74
993·5	3·61	7·92	991·3	4·94	10·81	989·1	6·35	13·87
993·4	3·67	8·05	991·2	5·01	10·96	989·0	6·42	14·02
993·3	3·73	8·18	991·1	5·07	11·09	988·9	6·49	14·17
993·2	3·78	8·29	991·0	5·13	11·22	988·8	6·55	14·30
993·1	3·84	8·42	990·9	5·20	11·38	988·7	6·62	14·45
993·0	3·90	8·55	990·8	5·26	11·51	988·6	6·69	14·60
992·9	3·96	8·68	990·7	5·32	11·64	988·5	6·75	14·73

The specific gravity of the spirit having been found to be 991·5, this by the table is equal to 10·55 per cent. of proof spirit,[1] or 4·82 per cent. of absolute alcohol by weight.

5. **Acidity.**—The acidity of beer is chiefly due to organic acids and acid phosphates.

It was formerly believed that the acidity of beer was due to acetic acid, and Graham, Hoffmann, and Redwood based their experiments on this assumption. It is now known, however, that newly brewed beer contains scarcely any acetic acid, although the acidity in original gravity determinations is still expressed in terms of percentage acetic acid. The percentage of acidity in a beer has a direct bearing upon the determination of the original gravity, since if acid exists it must have been formed at the

[1] Proof spirit has the specific gravity 0·9198, and contains 49·24 per cent. by weight and 57·06 per cent. by volume of alcohol.

expense of the alcohol or sugar, which will therefore be indicated too low by the original gravity experiment. Allowance for such contingency has been made by the investigators mentioned, who have published the following table, by which any excess of normal acidity may be calculated back to "spirit indication" and allowed for.

FOR ASCERTAINING THE VALUE OF THE ACETIC ACID.

Excess per cent. of Acetic Acid in the Beer.	Corresponding Degrees of "Spirit Indication."									
	·00	·01	·02	·03	·04	·05	·06	·07	·08	·09
·0		·02	·04	·06	·07	·08	·09	·11	·12	·13
·1	·14	·15	·17	·18	·19	·21	·22	·23	·24	·26
·2	·27	·28	·29	·31	·32	·33	·34	·35	·37	·38
·3	·39	·40	·42	·43	·44	·46	·47	·48	·49	·51
·4	·52	·53	·55	·56	·57	·59	·60	·61	·62	·64
·5	·65	·66	·67	·69	·70	·71	·72	·73	·75	·76
·6	·77	·78	·80	·81	·82	·84	·85	·86	·87	·89
·7	·90	·91	·93	·94	·95	·97	·98	·99	1·00	1·02
·8	1·03	1·04	1·05	1·07	1·08	1·09	1·10	1·11	1·13	1·14
·9	1·15	1·16	1·18	1·19	1·21	1·22	1·23	1·25	1·26	1·28
·10	1·29	1·31	1·33	1·35	1·36	1·37	1·38	1·40	1·41	1·42

The normal acidity of beer is assumed to be 0·10 per cent., expressed as acetic acid, and any larger amount is allowed for by the method mentioned.

For example, suppose we find by experiment that the beer contains an acidity equal to 0·18 per cent., expressed as acetic acid. The Inland Revenue allow in their calculation 0·1 per cent., therefore there is a remainder to be accounted for of 0·08 per cent. By referring to the table we see that 0·08 corresponds to 0·12 degree of "spirit indication," and this 0·12, added to our previous "spirit indication," = 8·6 (8·5 + 0·12), which by table gives 37·0 as against 36·5 originally found, which raises the original gravity before mentioned from 1060·5° to 1061°.

In order to determine the acidity in beer the process is as follows:—

One hundred c.c. of the beer at 60° F. (15·5° C.) are added to a porcelain dish and titrated with decinormal alkali, ascertaining the exact point of neutrality by means of delicate, newly prepared litmus paper.[1]

[1] It is preferable to filter the beer before titrating, and so get rid of the free CO_2, which would interfere with the litmus blue colour. The test with litmus should not be carried out in gaslight.

Suppose, for example, we have employed 17·5 c.c. $\frac{N}{10}$ alkali to effect neutrality; the calculation is then as follows:—

Each c.c. of $\frac{N}{10}$ alkali corresponds to ·009 gram lactic acid and ·006 gram acetic acid, but, as for original gravity purposes the acidity is reckoned as acetic acid, so we here express it in that manner; hence $17{\cdot}5 \times {\cdot}006 =$ **0·10 per cent. acetic acid**, which is normal, so that no correction is necessary for this particular beer in the original gravity determination. In analytical results, however, the acidity of a beer is usually expressed in terms of lactic acid, the $\frac{N}{10}$ alkali used being multiplied by ·009; thus $17{\cdot}5 \times {\cdot}009 =$ **0·157 per cent. lactic acid.**

Whichever way the acidity may be expressed, we have little or no knowledge as to the character of the acid-reacting substances present. It is not unusual, however, to express the acidity in terms of volatile and non-volatile acids. Thus the acidity of 100 c.c. of the spirit at the time of taking the original gravity is determined and expressed as volatile or acetic acid, a similar proportion of the residue being tested and expressed as non-volatile or lactic acid.

6. **Specific Rotatory Power.**—Filter a sample of the beer so as to rid it of carbonic acid gas, fill a 1 decimetre tube with the beer at 68° F. (20° C.), and polarise in the ordinary way.

Should the beer be too dark in colour, as it usually is with mild ales and always so with stout and porter, it becomes necessary to decolorise it, and this is best performed, according to Heron,[1] as follows:—

Take 100 grams of dry bleaching powder, treat in a mortar with about 200 c.c. distilled water until the whole is of a consistency of cream, and then filter. Now take a 100 c.c. flask, add to it 10 c.c. of this cream together with 50 c.c. of the beer, and allow to stand for 5 minutes. Now dilute to mark, mix, filter, and polarise in a 1 decimetre tube at 68° F. (20° C.). The reading × 2 gives the opticity of the beer, and this is calculated to 100 parts of dry solids.

Example.—Reading in a 1 decimetre tube = 2·8°. 2·8 × 2 = 5·6° on beer. The dry solids in the beer were found to equal 6·21 per cent.

$$6{\cdot}21 \times \frac{100}{5{\cdot}23} = [a]_{D\,3{\cdot}86} 118{\cdot}7^{\circ}.$$

[1] *Jnl. Fed. Inst. Brewing*, vol. i., 110.

7. **Colour.**—This is determined by Lovibond's tintometer, using a 1-inch cell for pale ales and a ½ inch cell for dark ales.

The tintorial power is expressed on the number of glass or glasses required to match the colour of the beer.

With pale ales the degrees (1 in cell) vary from 15° to 25°; with mild ales from 25° to 30°; and with dark beers (½ in. cell) from 35° to 50°.

There is usually a loss of about 5° in colour from the turning out of a copper to the collection of a beer in cask or bottle, so that a copper wort registering 30° would produce a beer showing about 25°.

With beers in much condition—bottled beers particularly—it is advisable to filter before filling the cell, as otherwise the escaping gas masks the true colour.

8. **Proteids or Albuminoids.**—These are determined in beer in precisely the same manner as with malt wort, using 10 c.c. of the beer and expressing the percentage on the dry solids (p. 208).

9. **Mineral Matter or Ash.**—Determined in the same manner as with malt wort, using 100 c.c. of the beer and expressing the result in percentage on the dry solids (p. 204).

10. **Iron.**—This is determined from the mineral matter or ash, as explained under Invert Sugar (p. 260).

11. **Salicylic Acid.**—In order to test whether a beer has been treated with salicylic acid, about 250 c.c. of the sample is evaporated to about 50 c.c., then transferred to a small flask, about 50 c.c. ether added, and the whole thoroughly shaken for a minute or so. The ether extracts the salicylic acid from the beer, and upon standing should separate as a clear liquid on the top of the solution. If it does not do so, a little more ether should be added, the solution again agitated, and allowed to stand. The solution is then evaporated over the water bath (not directly over the steam, and in no case over a Bunsen or other flame on account of the liability of the ether to catch fire). The residue is dissolved in a little water and one or two drops of ferric chloride solution added; a violet coloration proves the presence of salicylic acid.

12. **Malto-dextrins, etc.**—In order to ascertain the condition in which maltose and dextrin exist in a beer, and the proportion and type of the malto-dextrins which are present, Morris[1] some years ago drew up a satisfactory and complete method of analysis by which the low-type malto-dextrins are calculated from the

[1] *Jnl. Fed. Inst. Brewing*, vol. i., p. 125.

cupric reducing power and the optical activity before and after fermentation, the higher-type malto-dextrins and the stable dextrin being determined in a similar way as in malt wort (p. 221), the necessary corrections due to other bodies being made as described. Fermentation of the wort in the presence of cold-water malt extract removes the whole of the malto-dextrins and the stable dextrin, so that the cupric reducing power and optical activity of the residue after such fermentation is due to bodies other than maltose, dextrin, and malto-dextrins. The determinations and an example of the analysis are as follows:—

The original gravity is determined in the usual way, and the fermented matter calculated from the degrees of gravity lost corresponding to the "spirit indication."

The original reducing power of the beer is determined on from 3 c.c. to 10 c.c. of the beer, depending on the strength and age; 200 c.c. are then evaporated to about one-half to expel the alcohol, and the residue is made up to the original volume; it is then treated in the following manner:—

(*a*) *Degradation with Cold-water Malt Extract.*—Fifty c.c. are placed in 100 c.c. flask and 2·5 c.c. of cold-water malt extract are added; the mixture is then digested at 130° F. (55° C.) for 1 hour.

At the end of this time the liquid is boiled, cooled, and made up to 100 c.c. at 60° F. (15·5° C.). After filtering, the reducing power (in 5 or 10 c.c.) and the opticity are determined in the usual way. Fifty c.c. of the malt extract are digested side by side with the above, then boiled, cooled, and made up to 100 c.c. The reducing power (in 3 c.c.) and the opticity are then determined, and the result applied as a correction to the numbers obtained in (*a*).

(*b*) *Fermentation.*—In order to determine the low-type malto-dextrins remaining unfermented, a second 50 c.c. of the residue are set to ferment with about 0·25 gram of pressed yeast at about 75° F. (20° C.). When the fermentation is at an end, which it will be in from 48 to 72 hours, the liquid is cooled, a little alumina cream added, and the whole made up to 100 c.c. After filtration the reducing power is determined in the usual manner, in 5 or 10 c.c. of the filtrate.

(*c*) *Fermentation in the Presence of Cold-water Malt Extract.*—It is necessary to correct the numbers in (*a*) and (*b*) for the unfermentable and reducing substances present in the beer. In order to do this, 50 c.c. of the residue are fermented as in (*b*), but with the addition of 2·5 c.c. of cold-water malt extract. When the

fermentation is complete, the liquid is treated in all respects as in the last determination, and the reducing power and opticity are determined in the bright solution, 10 c.c being taken for the former. But the malt extract itself contains, as we shall subsequently see, these reducing and fermentable substances. It is therefore necessary, in order to arrive at an absolutely correct result, to ferment 50 c.c. of the extract side by side with the above; and after fermentation is at an end, make up to 100 c.c. and determine the reducing power and opticity of the residue.

The method of calculation is briefly as follows:—The low-type malto-dextrins are obtained by deducting the copper oxide after fermentation from the original reducing power, and calculating the difference into maltose. The combined maltose is obtained by deducting the reducing power, after fermentation with cold-water malt extract, from that after fermentation above. The combined dextrin is calculated from the increase in reducing power after degradation with malt extract. The free dextrin is found from the opticity after degradation, less that due to the total maltose and the unfermentable residue. And the unfermentable residue is obtained from the reducing power after fermentation with cold-water malt extract, the proper corrections for the malt extract itself being applied where necessary.

The following example of the numbers obtained with a pale ale illustrates the method:—

Original gravity, 1062·08; degrees of gravity lost, 42·64°.
Original reducing power, 2·578 grams CuO per 100 c.c.

(*a*) After degradation, 100 c.c. reduced 3·758 grams CuO, and the reading in a 100 mm. tube was 19·8 divisions; 5 c.c. of the malt extract gave 0·4830 gram CuO, and a reading in a 100 mm. tube of 8·0 divisions.

(*b*) After fermentation, 100 c.c. reduced 1·990 gram CuO.

(*c*) After fermentation in presence of malt extract, 100 c.c. reduced 1·096 gram CuO, and the reading in a 100 mm. tube was 1·8 divisions; the fermented malt extract gave a reduction of 0·0786 gram CuO per 5 c.c., and a reading of 0·8 division in a 100 mm. tube.

When the foregoing determinations are corrected as described above, we get:—

1.	Original reduction . .	2·578	grams CuO	per	100 c.c.
2.	Reduction after degradation	3·275	,,	,,	,,
3.	,, ,, fermentation	1·990	,,	,,	,,

4. Reduction after fermentation with addition of malt extract	1·017 grams CuO per 100 c.c.
5. Opticity after degradation .	19·4 divisions in 100 mm. tube.
6. " " fermentation with addition of malt extract	1·84 " " "

The reduction due to the unfermented, low-type malto-dextrins is then determined by deducting 3 from 1—

$$2{\cdot}578 - 1{\cdot}990 = 0{\cdot}588 \text{ gram CuO};$$

that due to the "amyloin-maltose" by subtracting 4 from 3—

$$1{\cdot}990 - 1{\cdot}017 = 0{\cdot}973 \text{ gram CuO};$$

that due to the maltose formed by degradation from the "amyloin-dextrin" by taking 1 from 2—

$$3{\cdot}275 - 2{\cdot}578 = 0{\cdot}697 \text{ gram CuO};$$

and the unfermentable, non-degradable residue is represented by 4. The free dextrin is obtained from 5 after deducting 6 plus the opticity due to the total maltose present after degradation (found by subtracting 4 from 2).

Calculating the foregoing quantities of CuO into maltose by the usual factor, we get—

0·438 gram unfermented, low-type malto-dextrins calculated as maltose,
0·724 " combined maltose,
0·517 " " dextrin calculated as maltose;

and multiplying by 0·95 to correct for the difference in molecular weight, we get—

0·491 gram combined dextrin,
0·756 " unfermentable reducing residue, calculated as maltose;

and using the usual factors, we get—

1·955 gram free dextrin.

These results are expressed as grams per 100 c.c. of beer; they are now calculated as percentages on the original wort solids, obtained from the original gravity, in this case 16,083 grams.

The results then are:—

Fermented matter	68·7 per cent.
Low-type malto-dextrins (calculated as maltose)	2·72 ,,
Combined maltose	4·51 ,,
,, dextrin	3·05 ,,
Free dextrin	12·16 ,,
Unfermentable reducing residue (calculated as maltose)	4·76 ,,

13. **Brilliancy.**—All beers should be perfectly brilliant and free from suspended bodies.

When a beer is not bright, some of the causes can be ascertained by the following methods:—

(1) Filter the beer through an ordinary filter paper. If the beer filters bright, the trouble is due either to the presence of

A—Normal yeast, or
B—Wild yeasts.

If the beer does not filter bright, proceed with test 2.

(2) Pour upon a plate a little of the beer, add a drop or two of iodine solution. If the beer gives a blue reaction, it is due to the presence of starch (C). If the beer does not give a starch reaction, proceed with test 3.

(3) Warm a sample of the beer in a test-tube or beaker. If it becomes brilliant, the trouble is due to the presence of glutinous matter or hop resin. To find which of these one has to deal with, proceed with test 4. If the beer remains cloudy, then proceed with test 5.

(4) Pour a little of the beer into a test-tube or beaker, and add a little ether. If the beer remains cloudy, the trouble is due to the presence of glutinous matter (E). If the beer becomes clear, the trouble arises from the presence of hop resin (F).

(5) Obtain a deposit from the beer by means of the centrifuge (fig. 37 or 38, p. 34 or 35), and examine the deposit under the microscope.

The trouble may thus be traced to the presence of *Saccharobacillus Pastorianus*, *Sarcina*, or other bacterial organisms; or to the presence of wild yeasts or an excess of normal yeast.

It often happens that the cause of the cloudiness in beer is due to a combination of simple causes, thus—wild yeasts combined with bacteria; starch combined with bacteria. In the cases of wild yeasts combined with bacteria, beer does not take finings, or

takes them very badly. In cases where starch exists the fermentation is very sluggish.

Sugar Analysis.

Invert-sugar—Raw sugars—Glucose—Priming syrups—Caramels.

Invert-sugar.

The following are the determinations to be made:—

1. Specific gravity of 10 per cent. solution.
2. Extract per cwt.
3. Mineral matter or ash.
4. Dry extract.
5. Moisture.
6. Proteids or albuminoids.
7. Colour.
8. Iron.
9. Acidity.
10. Invert-sugar.
11. Cane-sugar.
12. Specific rotatory power.
13. Unfermentable bodies.
14. Percentage composition of dry extract.

1. **Specific Gravity of 10 per cent. Solution;** and 2. **Extract per cwt.**—Carefully weigh 20 grams of the sugar in a small tared beaker or porcelain dish, add a little distilled water, and place on the water bath so as to thoroughly dissolve the sugar. Transfer the solution to a 200 c.c. flask, cool, make to mark at 60° F. (15·5° C.) with distilled water, and take the specific gravity by means of the specific-gravity bottle.

Example:—

$$\text{Specific gravity} = 1032{\cdot}10 - 1000 =$$

$$32{\cdot}10 \times \frac{112}{100} = \mathbf{35{\cdot}95 \text{ lbs. per cwt.}}$$

3. **Mineral Matter or Ash.**—Weigh 5 grams of the sugar in a tared platinum dish, add 1 or 2 c.c. of sulphuric acid, and ignite to ash as described under Malt Analysis (p. 204).

Example.—Five grams of sugar taken.

Weight of platinum dish + ash	60·708
Weight of platinum dish	60·631
	·077

$$0{\cdot}77 \times 20 = \mathbf{1{\cdot}54 \text{ per cent.}}$$

The ash may be used for the estimation of iron.

4. **Dry Extract**; and 5. **Moisture.**—Under the heading Solution Weight and Solution Factors (p. 57), it has been explained that the method of ascertaining the amount of solid carbohydrate matter present in a solution, by evaporating a known bulk to dryness and weighing the residue, is unsatisfactory; since it becomes necessary, in order to remove the last traces of moisture, to continue heating for a considerable time, and by thus heating, the organic substance is partially decomposed.

It has therefore been found preferable to ascertain the amount of matter in solution by taking the specific gravity and dividing the excess weight over water by a factor, the most generally adopted one being 3·86. Thus the specific gravity of our 10 per cent. solution was found to be 1032·10, so that $1032{\cdot}10 - 1000 = 32{\cdot}10$, which divided by the factor $3{\cdot}86 = 8{\cdot}316$, and this $\times$ 10 on account of it being a 10 per cent. solution $= 83{\cdot}16$ per cent. of solids.

These solids, however, are only apparent since some of them are due to the saline bodies contained in the sample. Heron has shown that the ash of sugars has a density approximately twice that of the carbohydrates (3·86), that is to say, the divisor is about 8. In other words, where 1 gram of sugar when dissolved in 100 c.c. of water gives approximately a specific gravity of 1003·86, 1 gram of the ash of the sugar, added to water and made up to 100 c.c., gives a specific gravity of 1008.

To be accurate, we should therefore make a correction for the saline bodies by dividing that portion of the specific gravity due to sugar by 3·86, and that due to mineral matter by 8·0. Having ascertained the amount of mineral matter present in the sugar, we may therefore either deduct the gravity due to this, or, what amounts to the same thing, after calculating the apparent solids by the use of the factor 3·86, we may make the correction as follows:—

The mineral matter is expressed too high as dry solids in the ratio of 8 to 3·86, we therefore multiply the ash by 8 and divide by 3·86, or we use the factor $2{\cdot}07\left(\frac{8}{3{\cdot}86} = 2{\cdot}07\right)$; then, deducting the result from the apparent solids, we obtain the solids due to the actual carbohydrate matter thus—

$\text{Ash} = 1{\cdot}22$ per cent. $\times\ 2{\cdot}07 = 2{\cdot}52$.
Apparent solids $= 83{\cdot}16 - 2{\cdot}52 = 80{\cdot}64$;
and $80{\cdot}64 + 1{\cdot}22 =$ **81·86 per cent. dry extract**, and
$100{\cdot}00 - 81{\cdot}86 =$ **18·14 per cent. moisture.**

6. **Proteids or Albuminoids.**—These are estimated by means of Kjeldahl's method, particulars of which are given under Malt Analysis, p. 207.

Example.—In titrating the distillate with $\frac{N}{20}$ ammonia, suppose the quantity required to neutralise the 75 c.c. of $\frac{N}{20}$ acid equalled 71·9 c.c. Then $75·0 - 71·9 = 3·1$ c.c. $\frac{N}{20}$ acid neutralised by the ammonia derived from the albuminoids originally in 5 grams of sugar, and $3·1 \times ·0007 = ·00217 \times 20 = ·04340 \times 6·33 =$ **0·27 per cent. albuminoids.**

7. **Colour.**—The colour of the 10 per cent. solution is taken by Lovibond's tintometer, using a 1 inch cell and expressing the result in percentage, *i.e.* multiplying the number of the glass or glasses used by 10, thus:—

10 per cent. solution required glass No. 3.
$3 \times 10 = 30$ degrees per cent.

The brilliancy of the solution should be noted.

8. **Iron.**—This may be detected and estimated where necessary from the 10 per cent. solution.

One hundred c.c. of the 10 per cent. solution are taken in a Nessler tube, a few drops of acetic acid added, and one or two drops of potassic permanganate. The presence of iron is indicated by the blue or greenish-blue coloration produced, and according to the intensity of the colour so is the quantity of iron present.

It is very unusual to find sugars containing more than a mere trace of iron, but should there be any excessive coloration, it is advisable to quantitatively estimate the amount thus:—

One hundred c.c. of 10 per cent. sugar solution taken in a Nessler tube, two drops of potassic ferrocyanide added.

One hundred c.c. of water taken in a Nessler tube, two drops of potassic ferrocyanide added, and a standard solution of iron (p. 137) run in from a burette until the colour of the tube containing the sugar solution is imitated.

Each 1 c.c. of the iron solution $= 0·1$ milligram of iron. The amount of iron solution required $= 0·2$ milligram, *i.e.* 0·2 mgm. per 10 grams of sugar, or $\times 10 = 2·0$ mgms. per 100 grams of sugar, or 0·002 per cent. It is usual to express the amount of iron as ferric oxide Fe_2O_3; 112 parts of

Fe = 160 parts of Fe_2O_3, so $\cdot002 \times \frac{160}{112} = 0\cdot28$ gram Fe_2O_3 per cent.

Instead of employing the sugar solution for the determination of iron, **the ash previously determined may be used for the purpose,** thus :—

The ash is dissolved by the addition of a little hydrochloric acid, the solution then evaporated to dryness to separate the silica, then again dissolved in hydrochloric acid and the iron precipitated by the addition of a little ammonia. The solution is now filtered, the precipitate containing the iron redissolved with hydrochloric acid, and the solution evaporated almost to a drop in order to get rid of the great excess of hydrochloric acid, which would interfere with the reaction later on. This small quantity of solution is then washed into a Nessler tube, made up to 50 c.c. with distilled water, and a few drops of potassic ferrocyanide added. A blue coloration takes place, and this coloration is matched in another Nessler tube by adding 50 c.c. of distilled water, about 1 drop of hydrochloric acid, and an amount of potassic ferrocyanide equal to that added to the previous Nessler tube. The standard solution of iron is then run in from a burette until the colour matches, and the calculation performed as described.

9. **Acidity.** — Occasionally some invert-sugars are purposely left faintly acid, as they are then of a paler colour. To determine the acidity, take 100 c.c. of the 10 per cent. solution in a porcelain dish and titrate with $\frac{N}{10}$ alkali, using litmus paper as indicator.

Example.—One hundred c.c. 10 per cent. solution required 7·5 c.c. $\frac{N}{10}$ alkali. Each c.c. of $\frac{N}{10}$ alkali = ·009 gram of lactic acid: $7\cdot5 \times \cdot009 = \cdot0675$ acid in 10 per cent. solution, or 0·67 per cent.

10. **Invert-sugar.** — Ten c.c. of the 10 per cent. solution are run into 100 c.c. flask and made to mark with distilled water = 1 per cent. solution.

A burette is filled with this 1 per cent. solution and Fehling's solution then prepared as follows :—

Twenty-five c.c. of copper sulphate solution and 25 c.c. alkaline tartrate are added to a beaker together with 30 c.c. of distilled water. The beaker is then placed in a boiling water bath, and when the contents have reached the boiling point, 20 c.c. of the

1 per cent. sugar solution are added and the whole boiled for exactly 12 minutes. The beaker is then removed and the contents filtered. A good filter paper must be used, or otherwise some of the CuO is liable to pass through. If such were the case it would, after standing, be found at the bottom of the beaker, in which case it becomes imperative to refilter. Wash the precipitate several times, using about 200 c.c. hot distilled water for the purpose, and finally wash with about 10 c.c. of strong alcohol; then dry the paper in the water oven, transfer to a tared crucible, and burn off; ignite to redness for 15 minutes, cool under desiccator, and when cold weigh, repeating the heating, cooling, and weighing until the weight is constant.

Example :—

Weight of crucible + CuO . . .	6·654 grams.
Weight of crucible	6·340 „
	0·314

20 c.c. of 1 per cent. solution gave ·314 gram CuO. ·314 × Fehling's factor ·4715 = ·1480 invert-sugar in 20 c.c., and $\cdot 1480 \times \frac{100}{200} =$ ·7400 invert in 100 c.c. of 1 per cent. solution or 1 gram of sugar. The sugar therefore contains 74·0 per cent. **invert**.

11. **Cane-sugar**. — Cane-sugar does not reduce Fehling's solution, we have therefore to invert it as follows :—

Ten c.c. of the 10 per cent. sugar solution are added to a 100 c.c. flask, 50 c.c. of distilled water added, together with 5 c.c. $\frac{4}{N}$ hydrochloric acid. The flask is then placed on the water bath and the contents maintained at a temperature of 150° F. (65·5° C.) for 20 minutes. The flask is then removed, the contents cooled to 60° F. (15·5° C.), neutralised by the addition of 5 c.c. $\frac{4}{N}$ alkali, made up to mark with distilled water, and thoroughly agitated.

We now have a 1 per cent. solution containing the original invert-sugar plus that produced by the inversion of any cane-sugar originally present. The increased proportion of invert-sugar now found in this solution gives us the amount of cane-sugar. We therefore carry out a Fehling's test with this 1 per cent. solution, identical with that previously carried out with the invert-sugar, and from the cupric oxide reducing power calculate the amount of total invert-sugar in the sample. By then de-

ducting one from the other and multiplying the increase of invert-sugar by ·95,[1] we arrive at the percentage of cane-sugar originally present.

Example :—

20 c.c. of 1 per cent. solution gave ·318 gram CuO.

·318 × Fehling's factor ·4715 = ·150 invert in 20 c.c.

$\cdot 150 \times \frac{100}{20} = \cdot 750$ invert in 100 c.c. 1 per cent. solution,

or 1 gram of sugar = 75·0 per cent.

In our former experiment we have invert-sugar = 74·00 per cent.; so 75·0 − 74·0 = 1·0 per cent. sugar due to cane, which × ·95 = **0·95 per cent. cane-sugar.**

Instead of carrying out the inversion of the sugar by the action of acid, yeast may be employed, thus :—

Ten c.c. of the 10 per cent. solution are added to a 100 c.c. flask, together with 20 c.c. distilled water, and digested with a small quantity of washed and pressed yeast (·5 to 1 gram) at 125° F. (51·6° C.) for 5 hours. The solution is then heated to expel alcohol, cooled to 60° F. (15·5° C.), a little alumina cream added, made up to 100 c.c. (= 1 per cent. solution), filtered, and the cupric oxide reducing power determined as described.

12. **Specific Rotatory Power.** — The optical activity of invert-sugar is levo-rotatory (−). If dextro-rotatory (+) the sugar either contains much cane-sugar or there is present an admixture of glucose, and the amount of such should be calculated from the specific rotatory power. Although, however, invert-sugar theoretically consists of equal parts of dextrose and levulose, commercial samples always contain an excess of dextrose, owing to a slight decomposition of the levulose during inversion. Allowance for this must be made when attempting to estimate the admixture of glucose.

Ten per cent. solution boiled and cooled to 68° F. (20° C.) examined by the polarimeter in a 1 decimetre jacketed tube. Specific rotatory angle = 1·26° × 10 $= [a]_D - 12\cdot60$ on sugar.

13. **Unfermentable Bodies.**—All sugars contain a proportion of unfermentable matters which have both a cupric reducing and an optical activity. It is usual to make a correction for these matters in the case of glucose, but this is hardly necessary

[1] The molecular weight of cane-sugar ($C_{12}H_{22}O_{11}$) = 342 ; that of invert-sugar ($C_{12}H_{24}O_{12}$) = 360. 360 parts of invert-sugar are produced from 342 parts of cane-sugar, hence $\frac{342 \times 100}{360} = \cdot 95$.

in the case of invert-sugar. For all purposes of commercial analysis the usual way is to calculate them by difference as follows:—

Total solids		81·86 per cent.
Less bodies determined—		
Invert-sugar	74·00	
Cane-sugar	0·95	
Albuminoids	·27	
Ash	1·54	
		76·76 ,,
Unfermentable bodies .		5·10

14. **Percentage Composition of Dry Extract.** — The determinations are made on a rule-of-three basis as follows:—

Dry extract . .	81·86.	
Mineral matter . .	$\frac{1{\cdot}54 \times 100}{81{\cdot}86} =$	1·89 per cent.
Albuminoids . .	$\frac{0{\cdot}27 \times 100}{81{\cdot}86} =$	0·33 ,,
Invert-sugar . .	$\frac{74{\cdot}0 \times 100}{81{\cdot}86} =$	90·39 ,,
Cane-sugar . . .	$\frac{0{\cdot}95 \times 100}{81{\cdot}86} =$	1·15 ,,
Unfermentable bodies .	$\frac{5{\cdot}10 \times 100}{81{\cdot}86} =$	6·24 ,,
		100·00

The full analysis works out as follows:—

INVERT-SUGAR.

1. Specific gravity of 10 per cent. solution	1032·10
2. Extract per cwt. (112 lbs.) . .	35·95
3. Mineral matter	1·54
4. Dry extract	81·86
5. Moisture	18·14
6. Proteids or albuminoids . . .	0·27
7. Colour	30°
8. Iron	0·2
9. Acidity	0·67
10. Invert-sugar	74·00
11. Cane-sugar	0·95
12. Specific rotatory power . . .	12·60°
13. Unfermentable bodies . . .	5·10

14. Percentage Composition of Dry Extract.

Invert-sugar	90·39
Cane-sugar	1·15
Unfermentable bodies	6·24
Proteids or albuminoids	·33
Mineral matter or ash	1·89
	100·00

Raw Sugars.

The determinations to be made with all raw sugars are the same as with invert-sugar, and are carried out in precisely the same manner.

Glucose Analysis.

The following are the determinations to be made :—

1. Specific gravity of 10 per cent. solution.
2. Extract per cwt.
3. Mineral matter.
4. Dry extract.
5. Moisture.
6. Proteids or albuminoids.
7. Colour.
8. Iron.
9. Acidity.
10. Cupric oxide reducing power.
11. Specific rotatory power.
12. Cupric oxide reducing power and specific rotatory power after fermentation.
13. Unfermentable bodies.
14. Percentage composition of dry extract.

The whole of these estimations, excepting No. 12, are determined exactly as with invert-sugar. It will therefore only be necessary to repeat the cupric oxide reducing power No. 10, and specific rotatory power No. 11, to make the determination of No. 12 explicit.

10. **Cupric Reducing Power.**—Seventeen c.c. of the 1 per cent. solution gave ·298 gram CuO, and ·298 × Fehlings factor ·4535 $= \cdot 1351 \times \frac{100}{17} = \cdot 7941$ dextrose in 1 gram of sugar. The total reducing sugars expressed as dextrose therefore = 79·41 per cent.

11. **Specific Rotatory Power.**—Determined from a 10 per cent. solution by polarimetric examination at 68° F. (20° C.) in a 1 decimetre tube.

Example.—Specific rotatory angle = + 5·20°. 5·20 × 10 = + 52° $[a]_D$ on sugar.

12. **Cupric Oxide Reducing Power and Specific Rotatory Power after Fermentation.**—One 100 c.c. of a 20 per cent. solution are placed in a 200 c.c. flask and the whole sterilised by boiling for a few minutes and then cooling to about 75° F. (23·8° C.).

Two to three grams of washed and pressed yeast are now added, and the mixture set on the forcing tray to ferment.

When the fermentation is complete, no further evolution of CO_2 being observed, the solution is boiled to expel the alcohol, then cooled and a little alumina cream added, and finally made to mark (200 c.c.) at 60° F. (15·5° C.) with distilled water and filtered.

The reducing power of this 10 per cent. solution gave ·293 gram CuO × 50 = 14·65 × Fehling's factor ·4535 = 6·64 *per cent. reducing power expressed as dextrose.*

The specific rotatory power of the fermented sugar solution, examined in a 2 decimetre tube at 68° F. (20° C.), gave a reading of 1·99° ÷ 2 = **9·95° on sugar.**

From these figures the maltose and dextrose proportions are derived, thus:—

Total sugars expressed as dextrose .	79·41	per cent.
Unfermented sugar expressed as dextrose	6·64	,,
Sugar removed by fermentation	72·77	

Total specific rotatory power	52·00°
Specific rotatory power after fermentation .	9·95
Rotatory power removed by fermentation	42·05

The maltose and dextrose having both been removed by fermentation, the loss in reducing and opticity is due to these substances, and from their representative figures we calculate the proportion of each of these bodies originally in the solution, thus:—

Maltose has a K 61 [1] and a specific rotatory power of $[a]_D$135·9°. Dextrose has a K 100 and $[a]_D$51·7°.

One part of dextrose therefore has an opticity of ·517°, and the reducing sugar, which has been calculated as dextrose, multiplied by this value, gives 72·72 × ·517° = 37·59°. Now, if our sugar were pure dextrose, the opticity would be 37·59°. The

[1] K equals the reducing power of a sugar compared with dextrose, the latter being taken as 100. Sixty-one parts of maltose precipitate the same amount of cupric oxide as 100 parts of dextrose, therefore its reducing power is expressed as K 61.

sugar, however, has an opticity of 42·05° or 4·46° above that required for dextrose, this excess opticity being due to the presence of a proportion of maltose, which has a higher opticity than dextrose. Maltose has an opticity of 135·9°, but so far we have expressed maltose present in terms of dextrose; we have therefore to ascertain the opticity which corresponds to maltose when thus expressed.

It will be seen that our maltose is expressed as dextrose, or K 100, and not as K 61; we have therefore to calculate the angle to correspond with this K 100, thus:—

$$135{\cdot}9^\circ \times \frac{100}{61} = [a]_D 222{\cdot}8^\circ.$$

From this figure we deduct the K 100 = $[a]_D 51{\cdot}7$, which gives us $[a]_D 171{\cdot}1^\circ$.

The rise of opticity in our sample is 4·46°, therefore

$$4{\cdot}46 \times \frac{100}{171{\cdot}1} = 2{\cdot}60.$$

Therefore:—

Total dextrose	72·77
Dextrose due to maltose	2·60
Dextrose in sample	70·17

The amount of maltose which the dextrose represents has now to be determined, thus—

The dextrose with K 100 is calculated to maltose K 61 as follows:—

$$2{\cdot}60 \times \frac{100}{61} = 4{\cdot}26 \text{ per cent. maltose.}$$

We now deduct the albumin and mineral matter from the residue after fermentation to obtain the dextrin, gallisin, and other bodies, which are usually grouped together, as their separate determination is unsatisfactory.

Example:—

Dextrose	70·17	per cent.
Maltose	4·26	,,
Albumin	·90	,,
Mineral matter	·86	,,
Moisture	16·78	,,
Dextrin, gallisin, unfermentable bodies	7·03	,,
	100·00	

Expressed as Percentage Composition of Dry Extract.

Dry extract	83·22	
Dextrose	$\frac{70{\cdot}17 \times 100}{83{\cdot}22} = 84{\cdot}31$	per cent.
Maltose	$\frac{4{\cdot}26 \times 100}{83{\cdot}22} = 5{\cdot}11$	„
Albumin	$\frac{0{\cdot}90 \times 100}{83{\cdot}22} = 1{\cdot}09$	„
Mineral matter	$\frac{0{\cdot}86 \times 100}{83{\cdot}22} = 1{\cdot}04$	„
Unfermentable bodies . .	$\frac{7{\cdot}03 \times 100}{83{\cdot}22} = 8{\cdot}45$	„
	100·00	

Priming Syrups.

These are prepared from either pure cane-sugar, invert-sugar, or glucose, and the determinations to be made are precisely as for invert-sugar, or for glucose, should the syrup be manufactured from the same, a point quickly ascertained by means of a polarimetric observation.

Caramel.

The following are the determinations to be made :—

1. Specific gravity of 10 per cent. solution.
2. Extract per cwt.
3. Dry extract per cent.
4. Moisture.
5. Mineral matter or ash.
6. Colouring power.
7. Deportment with beer and proof spirit.
8. Fermentability.
9. Cupric oxide reducing power.
10. Specific rotatory power.

Numbers 1, 2, 3, 4, 5, and 9 are determined as described with invert-sugar; numbers 6, 7, 8, and 10 being estimated as follows :—

6. **Colouring Power.**—The colour test, so far as caramels are concerned, is of exceptional importance, since the value of a caramel depends upon the tintorial power it is capable of yielding. Obviously, the greater the colouring capacity of a caramel, the

greater is its value, provided, of course, other conditions are normal.

It is best to make a ·01 per cent. solution by weighing 1 gram of the caramel in a small tared dish or beaker, adding a little water and raising to the boil so as to dissolve it, and then transferring the solution to a litre flask, cooling, and making to mark with water at 60° F. (15·5° C.). The brilliancy or opaqueness of the solution should be noted, and if cloudy, a little of it should be filtered through a dry filter paper before testing its colour value. The colour may then be ascertained by filling a 1 inch cell of Lovibond's tintometer, and the result expressed in degrees thus :—

Example.—·01 per cent. solution required glass No. 20 in 1-inch cell. Colour value = 20 degrees on ·01 per cent. solution.

7. **Deportment with Beer and Proof Spirit.** — The qualitative tests for a good caramel are solubility without cloudiness in proof spirit ; or, when added to pale ale in sufficient quantity to impart the colour of ordinary mild ale, the caramel should cause no deposit or cloudiness even after long standing. The latter is a very severe test ; but it is necessary because (*a*) a deposit indicates a disturbance of the carbohydrate equilibrium of the beer, and (*b*) when a deposit is found it not only consists of the caramel colouring matters, but carries down also some of the original colouring matter of the beer.

8. **Fermentability.**—A solution of the caramel, after boiling and cooling to 60° F. (15·5 C.), is made up to a specific gravity of 1050, 200 c.c. measured and added to a boiling flask, a little pressed yeast added, and the flask, after shaking, placed on the forcing tray.

When fermentation is complete the flask is removed and the contents boiled, the alcohol being thereby expelled. The solution is then cooled, made up to 200 c.c. at 60° F. (15·5° C.) with distilled water, filtered, and the specific gravity taken. The loss in specific gravity indicates the degrees of fermentability.

10. **Specific Rotatory Power.**—A solution of the boiled and cooled caramel, made to 1050 specific gravity, is decolorised as explained under Wort Analysis (p. 201) and examined by the polarimeter at 68° F. (20° C.). Good caramels show a low opticity.

Malt Culms or Rootlets.

Oil.—Determined as per prepared raw grain, p. 226.

Albuminoids.—Determined as per malt, p. 207.

PART VII.

ARSENIC.

As is well known, since the deplorable accidental contamination of beer by arsenical sugar in 1900, and the subsequent discovery by Thompson and Escourt of traces of arsenic in malt, it is now absolutely necessary to keep beer free from arsenic, at least to the extent recommended by the Royal Commission on Arsenic, the maximum being fixed at one-hundredth part of a grain per pound of malt or per gallon of beer.

MALT.

In the manufacture of malt, the barley when vegetated is dried off in the majority of cases by the direct heat evolved from the burning of oven coke, anthracite coal, or a mixture of the two, and these materials contain arsenic to a variable extent.

It is not unusual to detect amounts of arsenic varying from $\frac{1}{50}$th to $\frac{1}{200}$th part of a grain per lb. of these materials, and the question arises what quantity of the arsenic found in such materials is imparted to the malt.

Obviously the first point is the quantity of fuel used in drying off the malt, and this naturally varies with the system adopted and still more with the construction of the kiln. Roughly, however, it may be taken to vary from $\frac{1}{7}$th to $\frac{1}{4}$th of the weight of malt it has to dry.

Secondly, it must be borne in mind that if sulphur is mixed with the fuel the so-called fixed arsenic may become volatile; this, however, is negligible, because it is rarely that any considerable amount of sulphur is used intermixed with the fuel, the practice in burning sulphur, if at all resorted to, being to burn it separately.

Taking it that the fuel contains $\frac{1}{50}$th of a grain of arsenic per lb., and assuming that the whole quantity is evolved and retained in the malt, the latter will then show from $\frac{1}{350}$th to $\frac{1}{200}$th of a

grain per lb. Experience shows, however, that only about half the quantity of arsenic in the fuel finds its way into the malt, partly because a considerable fraction of it settles out on the walls, arches, and baffle-plates before it reaches the grain, and partly because some of it remains behind in the ash.

Under these circumstances, a malt dried over fuel containing so high an arsenic content as $\frac{1}{50}$th of a grain per lb. would be likely to show from $\frac{1}{700}$th to $\frac{1}{400}$th of a grain of arsenic per lb., according to the amount of fuel used per quarter of malt. It therefore appears that the question of malt contamination by arsenic is a very much overrated one, and that it is indeed seldom, and in fact only in very rare instances, that a malt is found to contain more than $\frac{1}{350}$th part of a grain per lb. Nevertheless, in view of the lesson taught in 1900, brewers and maltsters cannot be too careful, and all fuel used for drying malt should at any rate certainly not contain more than the $\frac{1}{50}$th of a grain per lb., and all malt produced or purchased should not contain more than the $\frac{1}{350}$th part of a grain of arsenic per pound.

Hops.

In the drying of hops the quantity of fuel used is different to that in drying malt. It may be taken as a fact that for every unit weight of hops an equal weight of fuel must be used in drying them, and assuming that all the arsenic in fuel (reckoning the fuel to contain so high a rate as in the former case, viz., $\frac{1}{50}$th grain per lb.) got into the hops, the hops will show $\frac{1}{50}$th of a grain of arsenic per lb. as against $\frac{1}{350}$th to $\frac{1}{200}$th in malt. One has therefore to be very careful with regard to the arsenic content of hops, since the construction of the oast-house kiln and the conditions of drying, as well as the large amount of fuel used, all tend to allow more of the arsenic content of the fuel to pass into and be retained by the hops, it being in fact roughly estimated that at least $\frac{2}{3}$rds of the arsenic content of the fuel are retained by the hops. Even so, however, the amount of hops used in both copper and cask in the manufacture of beer is small compared with that of malt, so that any hops showing even so high a quantity of arsenic as the $\frac{1}{50}$th of a grain per lb. are sufficiently free from arsenic for all purposes, and in comparison with malt showing such amounts as previously mentioned, would not even then bring the beer within the Royal Commission's limit of $\frac{1}{100}$th part of a grain per gallon. It is safest, however, when purchasing hops, to either obtain a guarantee that the hops do not contain more than the $\frac{1}{100}$th

part of a grain per lb., or to test the same, rejecting those that contain more.

Sugars.

The amount of arsenic commonly occurring in sugars varies from $\frac{1}{100}$th to $\frac{1}{200}$th part of a grain per lb., and the majority are now almost free. At any rate, provided they do not contain more than $\frac{1}{100}$th part of a grain per lb., they are sufficiently pure in this respect for all purposes.

It should be remembered, if using sulphuric acid as a converting agent in cases where cane-sugar is inverted by acid on the brewery premises, that the sulphuric acid employed is of the purest character and almost free from arsenic. As a rule the acid will be found to contain quantities of from 0·2 to 0·4 per cent., and such quantities may be ignored; but anything beyond 0·4 per cent. should result in the rejection of the acid for a purer supply.

Beer.

The surest test of all is that of the beer, and it is now the usual practice in numerous breweries for the analyst to test, for arsenic, every beer as racked, the detection of anything approaching the $\frac{1}{100}$th part of a grain of arsenic per gallon being sufficient to arouse suspicion and result in a complete analytical survey, in this direction, of all materials, such as water, hardening ingredients, malt, hops, sugars, antiseptics, yeast foods, and finings. By these means an excessive quantity of arsenic in any of these materials is quickly located, and means immediately taken to reduce the evil and once more come back to safe limits.

Arsenic Tests.

Reinsch Test.—Of all tests this is the most simple, but is absolutely useless for very minute quantities. It is performed as follows:—

About 200 c.c. of the beer, wort, or other solution, or about 2 grams of a solid substance ground to powder and mixed with about 200 c.c. of distilled water, are placed in a porcelain dish together with about 20 c.c. of pure hydrochloric acid. A small strip of polished copper foil is then added and the mixture boiled for a short time. If arsenic is present, the surface of the copper foil will become coated with a dark-grey film of As_2Cu_5.

The strip of copper is now removed from the dish, carefully

dried, and then folded and placed in a warmed, drawn-out glass tube.[1] The tube is then heated very slowly over a small Bunsen flame, when a white crystalline sublimate of As_2O_3 will form on the drawn-out portion of the tube. This white sublimate is known as the "mirror."

By making a standard solution of arsenious oxide and adding a definite quantity to a given bulk of acid solution, and boiling the same with a strip of copper, the arsenic in the solution will be deposited on the copper; and upon drying the copper and subliming it in a drawn-out glass tube as previously stated, a standard mirror is obtained.

By increasing or decreasing the strength of the arsenical solution, and repeating the experiment, a further standard mirror is obtained, and so on, so that numerous standard mirrors may be prepared. After sublimation the drawn-out portion of each glass tube is broken off and both ends sealed by the blow-pipe. The tubes are then attached to strips of cardboard, and the quantity of arsenic which each mirror represents is written thereon.

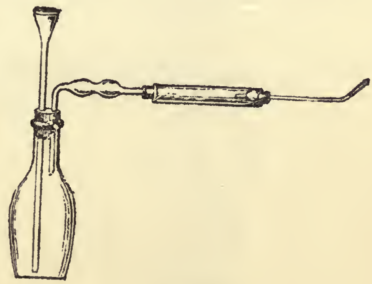


Fig. 77.

If we take one of these tubes and examine it under the microscope, employing for preference a $\frac{1}{16}$-inch objective, transparent, colourless, regular octahedra and tetrahedra crystals will be seen.

The mirror obtained from the sublimation of the copper employed for testing our beer, wort, or other substance is now compared with the standard mirrors, and the amount of arsenic calculated to percentage from the original quantity of substance or solution employed.

Marsh Test.—This is a far more reliable test than the Reinsch, and is performed as follows:—

Fit a 4-ounce flask with a thistle funnel and bent delivery tube as shown (fig. 77). Attach to the latter, by means of a rubber joint, a tube of hard glass about 4 inches in length and drawn out at the end to small bore, and support this on a ring of a retort-stand. Cover the bottom of the flask with granulated zinc, free from arsenic, add a little water, and then pour in through the funnel tube a little strong hydrochloric acid.

After hydrogen has been evolved and expelled all the air from

[1] Tubes of even bore and ready drawn may now be purchased.

the flask, there will be no risk of an explosion occurring when the gas is lighted; but it is well to cover the flask with a cloth before kindling the hydrogen. Now kindle the hydrogen and pour the solution to be tested into the flask through the thistle funnel. Press down upon the flame the inside of a clean dry porcelain dish, when a dusky black film of As may be deposited upon the cool surface.

Instead of employing a porcelain dish, the drawn-out tube may be heated by the tip of a small Bunsen flame as shown in fig. 79, when the arsenious oxide evolved will be sublimed, forming a mirror, as in our previous experiment.

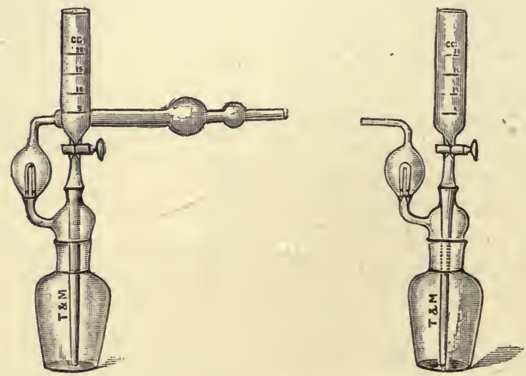


Fig. 78.

The tube is then broken off, both ends sealed with the blowpipe, and the mirror compared with the previously prepared standard mirrors, the amount of arsenic in the quantity of substance dealt with being thus estimated.

It is as well to mention that in both the experiments the dark-grey colours produced on the copper in the Reinsch test, and the black deposit produced on the porcelain dish in the Marsh test, may be due to antimony and not arsenic. To test whether this is so or not, a little freshly prepared solution of bleaching powder, containing calcium hypochlorate ($CaCl_2O_2$), poured upon the copper or black film on the dish, will rapidly dissolve and remove arsenic, whilst it has no action on antimony. The mirror produced in both cases may be recognised by its solubility in $CaCl_2O_2$, and by the crystals seen on microscopical examination.

Instead of employing an ordinary boiling flask as shown in fig. 77, it is now more usual, and certainly safer, to employ a Tyrer Marsh flask, different forms of which are shown (figs. 78 and 79).

These flasks consist of three separate pieces: the flask which holds the substance or solution being experimented with, the stopper with bulb connection, and the thistle funnel or stop-cock graduated tube. A few pieces of granulated zinc are added to the flask together with a little distilled water, the bulb stopper is then inserted, and then the thistle funnel or graduated tube. Acid is now added through the funnel or from the graduated tube, and upon coming in contact with the zinc hydrogen is set free. The gas passes up through the side tube in the stopper, circulates through the inner loop into the bulb, and from there makes its escape. Upon lighting the hydrogen, after all the air is expelled, and proving that the zinc and acid are free from arsenic, the light

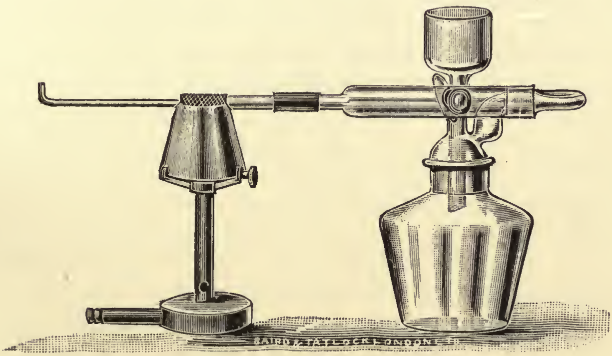


FIG. 79.

is put out and the stopper removed; after which the substance to be tested is added and the experiment continued, the mirror produced, if arsenic is present in the substance, being obtained and compared with standard mirrors in the manner already described.

Marsh-Berzelius Test.—This is carried out precisely as described with the Marsh test, excepting that a slight modification is introduced with regard to the apparatus. The modification consists in employing a drying tube, that is to say, a tube containing calcic chloride (so that the hydrogen evolved is free from moisture); and a roll of filter paper (previously soaked in a strong solution of lead acetate and then dried), so that any sulphuretted hydrogen evolved may be absorbed.

The following method of estimating arsenic, and in which the Marsh-Berzelius apparatus is employed, is the most reliable and accurate for the purposes of the brewer's analyst.

Apparatus.—The apparatus required for the test consists as shown (fig. 78 or 79) in a flask of about 50 c.c. capacity fitted with either a graduated tube, thistle funnel, or funnel and stop-pipe, the tube of which passes through the bulb stopper of the flask.

From the tube leading from the bulb stopper of the flask a drying tube is fitted by means of a piece of rubber tube. This tube is loosely plugged at both ends with cotton wool, and the centre portion filled with calcic chloride; a roll of filter paper (previously soaked in lead acetate and then dried) is also placed at one end of the tube. The object of the chloride of calcium is, as previously described, to dry the gas, that of the roll of filter paper to absorb any traces of sulphuretted hydrogen which may be evolved.

A piece of glass tube 200 mm. long, $4\frac{1}{2}$ mm. in internal, and $6\frac{1}{2}$ mm. external, diameter, is softened in the blowpipe flame and drawn out from the middle for about 30 mm. and again drawn out at one end to about 70 mm.; the diameter at the beginning of the drawn-out portion in the middle of the tube (for receiving the mirror) being about 2 mm. in internal diameter.

This tube is now attached to the end of the drying tube connected with the flask, and is supported in position by resting on a ring of a retort-stand.

A piece of fine iron gauze 20 mm. wide is wrapped round the tube at the point shown in fig. 79, and heated by a Bunsen flame. This is more satisfactory than applying the flame directly to the tube, and conduces to the formation of more uniform mirrors.

The Bunsen flame should be about 4 inches long, and protected till near the point by a conical chimney. The tube should be heated about half an inch from the shoulder at the point shown.

Beer.—Take 50 c.c. of the sample in a 200 c.c. Jena glass flask and evaporate to a syrup on a sand bath. Add 25 c.c. strong nitric acid and 5 c.c. strong sulphuric acid; place on the sand bath, having taken away the flame; allow the first violent action to subside, the acid fumes from which may be drawn away through a glass tube in the mouth of the flask by a water Sprengel pump through a solution of caustic soda in a Wolff's bottle. Then apply a Bunsen flame to the sand bath, and evaporate till the liquid begins to darken. When this occurs, add strong nitric acid in quantities of 3 c.c. at a time (the total quantity of nitric acid required varies from 30 to 50 c.c., depending on the quantities of organic matter present), until on further heating it continues colourless, and fumes strongly of sulphuric acid; cool,

dilute with 10 c.c. of water, and boil down to break up the nitro-sulphuric acid formed. When cold, dilute with 10 c.c. of water and deliver into the apparatus.

Testing Reagents.—A blank on the reagents and apparatus used should be made by boiling down 100 c.c. HNO_3 and 5 c.c. H_2SO_4 till all nitric is expelled, diluting and boiling down (this procedure removes every trace of nitric or nitrous acid), again diluting, and testing in the apparatus as above described.

Malt, Sugar, Caramel, Hops, Yeast, etc.—Take 5 grams of malt or other solid organic substance, add 25 c.c. HNO_3, and heat gently till the first violent action is over; then add 5 c.c. sulphuric acid and proceed as for beer (a total of from 50 to 75 c.c. of nitric acid will be required).

The Marsh-Berzelius apparatus (50 c.c. flask) should contain about 20–25 grams zinc. The $CaCl_2$ in the drying tube should be renewed as soon as the first few pieces become wet. Action is started by adding 5 c.c. of dilute sulphuric acid (10 parts concentrated sulphuric, 20 of water, and 1 part of a 10 per cent. solution of pure crystallised copper sulphate). Allow the evolution of gas to go on (the exit tube for the hydrogen being heated in the usual manner by means of a small Bunsen flame) until the hydrogen nearly ceases to be evolved; then fill up the tube and funnel of the Marsh-Berzelius apparatus with the solution previously treated as above, and allow the whole to run in, if only a very minute quantity of arsenic is supposed to be present; or run in an aliquot part if a larger quantity is supposed to exist. In about 15 minutes the flask is washed with 5 c.c. more of the above acid, which is added to the hydrogen flask in small quantities at a time to keep up the evolution of gas for a total of from 30 to 35 minutes after the first introduction of the previously treated beer, by which time, with this sized apparatus, all the arsenic will have passed off.

The hydrogen flame at the end of the drawn-out portion should be about 2 mm. long, and maintained as constant as possible: too slow a flow almost invariably gives double mirrors; too fast a flow gives irregular ones, difficult to compare with the standards.

Zinc.—Twenty grams of zinc should be tested by the action of 30 c.c. of dilute H_2SO_4—one of acid to two of water—containing a little copper sulphate to start the reaction; there should be absolutely no trace of arsenic mirror on the drawn-out portion of the glass tube.

Another experiment should then be made, adding a minute quantity of arsenic, say, equal to $\frac{1}{500}$th of a grain per gallon (when

working on 50 c.c.), equal to 0·029 part per 1,000,000, or an actual weight of 0·00143 mgrm., and compared with a standard tube to make sure that the zinc contains nothing which will hold back minute quantities of arsenic.

Standard mirrors for comparison are made by introducing into the apparatus known quantities of arsenic, say, commencing with $\frac{1}{50}$th of a grain per gallon when working on 50 c.c. A convenient method of preparing the standard mirrors is to employ a solution of arsenious acid containing 0·007145 of a mgrm. of As_2O_3 per 1 c.c. One c.c. of this solution is equal to $\frac{1}{100}$th of a grain of As_2O_3 per gallon when using 50 c.c., or $\frac{1}{100}$th of a grain per lb. when using 5 grams of material. This solution is suitable for standards between $\frac{1}{50}$th and $\frac{1}{100}$th of a grain per gallon, and a solution of $\frac{1}{10}$th this strength may be employed for the standard mirrors between $\frac{1}{100}$th and $\frac{1}{1000}$th of a grain per gallon.

The mirrors which will be found most useful are $\frac{1}{50}$th, $\frac{1}{66}$th, $\frac{1}{88}$th, and $\frac{1}{100}$th of a grain per gallon, corresponding to 2·0, 1·5 1·25, and 1·0 c.c. of the stronger solution; and $\frac{1}{111}$th, $\frac{1}{125}$th, $\frac{1}{143}$rd, $\frac{1}{166}$th, $\frac{1}{200}$th, $\frac{1}{250}$th, $\frac{1}{333}$rd, $\frac{1}{500}$th, $\frac{1}{666}$th, and $\frac{1}{1000}$th of a grain per gallon, corresponding to 9·0, 8·0, 7·0, 6·0, 5·0, 4·0, 3·0, 2·0, 1·5, and 1·0 c.c. respectively of the weaker solution.

Preservation of the Mirrors.—When the test has been completed, the point of the drawn-out portion of the glass tube is sealed by the blowpipe flame and also the other end of the tube. Without sealing, the tubes will keep for many months; but as it is possible that the mirrors may fade by exposure to light and air, they are, under the circumstances, best sealed and preserved in an atmosphere of hydrogen. Lastly, the importance of all reagents being absolutely free from arsenic cannot be overrated. Nitric acid can easily be obtained absolutely free from arsenic, and so also can zinc, as it is now manufactured electrolytically and freed from iron by treatment with acid for the purpose. It is advisable, however, with both hydrochloric and sulphuric acids, to dilute them with about $\frac{1}{3}$rd water, to add about 1 gram per litre of chromic acid, and distil. With sulphuric acid this operation suffices to free it from any trace of arsenic or sulphurous acid, whilst with hydrochloric acid a current of filtered air passed through the distillate for about 2 hours, by means of a water vacuum-pump, removes every trace of chlorine, and no trace of arsenic will be found in the product.

PART VIII.

INTERPRETATION OF THE RESULTS OF ANALYSIS.

Water.

The analysis of water, as will have been seen, is not itself a difficult matter, but great manipulative skill is required, on the one hand, in order to obtain reliable results, and a thorough knowledge of the subject of water supply is requisite on the other in order to discriminate as to the difference between a safe and an unsafe, a pure and an impure, supply.

The analyst on the brewery premises has not only to deal with the town or well water supplied to or at the particular brewery in which he is engaged, but with supplies of all kinds usually forwarded by the tied or other customers from sources wide apart. He has, therefore, to be in a position not only to determine the purity or otherwise, and the saline constituents, but to have sufficient knowledge of his subject to be able to state with good reason the nature of the strata through which the water has percolated, or whether it is an upland surface water, pure, and likely to remain so, or whether, on the other hand, it is open to contamination, and hence to be discarded or looked upon with suspicion. These are points, like most others pertaining to analyses, which can only be determined by an analyst possessing a wide and extensive knowledge, such, as far as water is concerned, as geology and physics. Besides these, the analyst must know how to artificially treat different waters in order to render them suitable for brewing different classes of beer; hence all that can reasonably be done in short space is to deal briefly with a subject that in itself would occupy a good-sized volume.

The primary form of natural water is rain, the chief impurities in which are traces of organic matter and ammonia derived from the atmosphere. On reaching the ground it becomes more or less

charged with the soluble constituents of the soil, such as calcic and magnesic carbonates, potassic and sodic chlorides, and other salts, which are dissolved, some by a simple solvent action, others by the agency of carbonic acid in solution. Draining off from the land, it will speedily find its way to a stream which, in the earlier part of its course, will probably be free from pollution by animal matter, except that derived from any manure which may have been applied to the land on which the rain fell. Thus, comparatively pure, it will furnish to the inhabitants on its banks a supply of water which, after use, will, in most instances, be returned to the stream in the form of sewage charged with impurity derived from animal excreta, soap, household refuse, etc., the pollution being perhaps lessened by submitting the sewage to some purifying process such as land irrigation, filtration, or clarification.

The stream in its subsequent course to the sea will be in some measure purified by slow oxidation of the organic matter, and by the absorbent action of vegetation, but not to any great extent.

Some of the rain will not, however, go directly to a stream, but sink through the soil to a well. If this be shallow, it may be considered as merely a pit for the accumulation of drainage from the immediately surrounding soil which, as the well is in most cases close to a dwelling, will be almost inevitably charged with excretal and other refuse, so that the water when it reaches the well will be contaminated with soluble impurities thence derived, and with nitrites and nitrates resulting from their oxidation. After use the water from the well will, like the river water, form sewage, and find its way to a river, or again to the soil, according to circumstances.

In the case of a deep well from which the surface water is excluded, the conditions are different. The shaft will usually pass through an impervious stratum, so that the water entering it will not be derived from the rain which falls on the area immediately surrounding its mouth, but from that which falls on the outcrop of the previous stratum below the impervious one just mentioned; and the water of the well will probably be entirely free from organic impurity or products of decomposition. But even if the water be polluted at its source, still it must pass through a very extensive filter before it reaches the well, and its organic matter will probably be in great measure converted by oxidation into bodies in themselves innocuous.

This is very briefly the general history of natural waters, and

the problem presented to the analyst is to ascertain, as far as possible, from the quality and quantity of the impurities present, the previous history of the water, and its present condition and fitness for the purpose for which it is to be used.

It is impossible to give any fixed rules by which the results obtained by the foregoing method of analysis should be interpreted. The analyst must form an independent opinion for each sample from a consideration of all the results he obtains.

The following classification as to the order of excellence of waters from different sources is given by Frankland:—

Pure	1. Spring water. 2. Deep-well water. 3. Upland surface water.
Suspicious	4. Rain water. 5. Surface water from cultivated lands.
Impure	6. River water to which sewage water gains access. 7. Shallow-well waters.

Free and Albuminoid Ammonia.—In the method of analysis detailed, Wanklyn endeavours to determine the amount of organic nitrogen contained in a water by converting it into ammonia and estimating it as such.

In judging by this process he classifies waters as follows:—

Class 1. Waters of extraordinary organic purity: those yielding from ·00 to ·05 part of albuminoid ammonia per million.

Class 2. Safe waters: those yielding from ·05 to ·10 part of albuminoid ammonia per million.

Class 3. Impure waters: those yielding more than ·10 part of albuminoid ammonia per million.

1. If the free ammonia exceeds ·08 part per million, recent contamination by urine is indicated. In such case a great excess of chlorides may be expected.

2. If the free ammonia and chlorine are present in only small quantity, and if the albuminoid ammonia comes off slowly on distillation, vegetable contamination is indicated.

3. Unless the albuminoid ammonia exceeds ·05 part per million, a water may be regarded as pure, even though the proportion of free ammonia and chlorine are large.

4. If free ammonia is present in only small amount, the albuminoid ammonia is not to be regarded as a reason for condemning a sample, unless it reaches ·10 part per million.

5. If, however, albuminoid ammonia reaches ·10 part per million, the water is suspicious, apart from the absence of free

ammonia and chlorine; whilst if the albuminoid ammonia exceeds ·15 part per million, the sample is to be absolutely condemned.

There are few chemists who now altogether accept the whole of the above statements, since Wanklyn appears to regard vegetable contamination almost as serious as animal pollution, whereas it is now generally known that the albuminoids derived from animal sources are much more dangerous than those derived from vegetable sources, the reason being that the former are much more putrescible (*i.e.* easily decomposable by bacteria) than the vegetable albuminoids.

It has been stated that by Wanklyn's method the *total* amount of nitrogen of the organic matter is never obtained as ammonia; that of the various nitrogenous organic substances submitted to his process some give up the whole of their nitrogen as ammonia, others two-thirds, some one-fourth, some very little, and others none whatever. Wanklyn claims, however, that many of the substances which yield as albuminoid ammonia such varying percentages of their total nitrogen, never occur in water at all, and that, adopting the standards he forms, a just judgment of a water may be always made. This is certainly true, and the process has become one universally employed by chemists, particularly as it is less complicated and difficult than others. In any case a bad water cannot escape the test.

Oxygen absorbed. — This test, originally devised by Forschammer, and known as the "oxygen or Forschammer process," was modified by Tidy, to whom we are indebted for its present application. It is a valuable confirmatory test, and from the amount of oxygen absorbed it is possible to obtain very useful information as to the contamination of a water.

Not only organic matter, but nitrates, ferrous iron, and sulphuretted hydrogen also absorb oxygen from potassic permanganate, and, as described, corrections for these must be made by a blank experiment.

Tidy gives the following standards for the valuation of water by this test, expressing the opinion that "the oxygen absorbed roughly represents one-tenth of the organic matter present."

1. Waters of great purity, oxygen not to exceed ·05 part per 100,000.

2. Waters of medium purity, not to exceed ·15 part per 100,000.

3. Waters of doubtful purity from ·15 to ·21 part per 100,000.

4 Impure water more than ·21 part per 100,000.

Tidy particularly protests against the drawing of any hard-and-fast lines, and gives the classification merely as a suggestion.

For domestic purposes the quantity should not exceed 0·05 part per 100,000, but anything under 0·25 part per 100,000 in a water for brewing purposes may be ignored.

Nitrates. — The ammonia derived from the atmosphere or from the putrefaction of organic matter is rapidly oxidised into nitrous and nitric acids, which combine with one or other of the alkalies or alkaline earths present in the soil to form corresponding salts. Consequently, although there are several reliable methods for estimating nitrites and nitrates, there are two ways of stating the quantity of the latter found on analysis. The declaration may be as "nitric acid"—really nitric anhydride N_2O_5—or as nitrogen existing as nitrates. When stated in the former, the amount will appear much larger than when stated in the latter way. The ratio between the two is 14 to 54; consequently, nitrogen as nitrates × 3·86 = nitric acid, and nitric acid ÷ 3·86 = nitrogen as nitrates. Amounts respectively expressed in grains per gallon nitric acid are as follows:—

Nitrates.	Nitric Acid.	Nitrates.	Nitric Acid.
0·10	0·386	1·30	5·018
0·15	0·579	1·40	5·404
0·20	0·772	1·50	5·790
0·30	1·158	1·60	6·176
0·40	1·544	1·70	6·562
0·50	1·930	1·80	6·948
0·60	2·131	1·90	7·334
0·70	2·702	2·00	7·720
0·80	3·088	2·10	8·166
0·90	3·474	2·20	8·492
1·00	3·860	2·30	8·878
1·10	4·246	2·40	9·264
1·20	4·632		

Pure waters from different sources contain various amounts of nitric acid; spring water contains on an average 2 grains per gallon and river water 0·57 grain per gallon. A water for domestic purposes should not contain more than 2 grains per gallon. For brewing purposes a soft water may contain $2\frac{1}{2}$ grains per gallon, but a hard water may contain up to 4 grains per gallon; beyond this, however, difficulties with the yeast, erratic fermentations, highly coloured beers and frequent instability of

both yeast and beer, as well as decidedly small outcrops of the former, is invariably found.

The only remedy against the action of any excessive amount is vigorous and rapid fermentations, low temperatures, and an excess of yeast. Finally, however, it must be borne in mind that the presence of nitric acid in a water may not be due to the oxidation of organic matter, but to the strata through which the water has percolated.

In certain districts nitrates exist in the strata, as already stated, as natural salts, and not as the oxidation products of contamination. Thus in some of the very pure waters of Kent, drawn from great depths, to which sewage cannot possibly have access, considerable quantities of nitric acid are found; as much as 4 to 5 grains per gallon being not unusual. The same thing occurs in certain waters drawn from the new red sandstone, in some of the gypsum pockets of which nitric acid undoubtedly exists to the extent of 2 to 6 grains per gallon.

Nitrites.—A water showing the presence of nitrites should always be looked upon with the greatest suspicion, as in most cases they indicate that organic matter is obtaining access to the well in quantities beyond those which the adjacent soil, acting in its capacity as a natural filtering bed, can convert into the more harmless nitrates. Nitrites have been found in deep well waters by some chemists; but the author has never yet discovered them in such waters, and is of opinion that their presence has been due more to the analytical method adopted than to the fact of their existence.

Chlorine.—When nitric acid is found in excessive quantity in a water, there is usually a large amount of chlorine also present. In the neighbourhood of the sea, however, large amounts of chlorine may exist without being an indication of organic pollution. A good instance of this is found in the supplies available for brewing at Yarmouth and other towns close to the coast. This is due to the atmosphere being laden with sea salt, which is absorbed by the rain, and thence passes into the water supplies; or again, it may arise from the fact that sea water has percolated through the soil and thus come into direct contact with the liquor supplies. At Durham, near the coast, the well waters have often been found to be distinctly saline owing to this percolation. But the general rule on this matter runs as follows:—If "isochlors" are drawn—that is to say, lines connecting wells of equal depth containing the same amount of chlorides—it is found that, excluding sewage contamination, the

further they are situated from the sea the less chlorine do the wells contain. Some years ago De Chaumont pointed out the influence of high and low tides on the proportion of chlorides in a well near a tidal river. He analysed samples taken from a well 83 feet deep and situate 2250 feet from the nearest point of a river in Hampshire. The chlorides fluctuated according to the state of the tide, ranging from 2·45 grains per gallon at low water to 2·8 grains per gallon at high tide. In its passage through strata containing sodium chloride, water may, obviously, take up large quantities of chlorides. The triassic clays of Cheshire, Worcestershire, and other places are rich in deposits of rock salt, and wells may there be sunk into springs of brine. And with these deposits of rock salt one often finds associated the chlorides of magnesium and potassium, besides frequent occurrence of gypsum.

Urine, which contains nearly 1 per cent. of sodium chloride, is a frequent source of pollution; consequently, a water containing a much larger amount of this salt than other waters occurring in the same district, and which are known to be uncontaminated by sewage, is to be regarded with suspicion. A fairly large amount of a highly nitrogenous substance called "urea" is also present in urine, and this, under the influence of bacteria, is rapidly transformed into ammonium carbonate; consequently, waters directly polluted by urine contain considerable amounts of free ammonia as well as chlorides. The drainage from sewers, middens, and cesspools are rich in both chlorine and ammonia, and as the latter is also one of the chief compounds derived from the decomposition of animal organic matter, its presence in the water of shallow wells often points to recent pollution with organic matters of animal origin. It will thus be seen that in judging the organic contamination of water the whole of the foregoing data must be conjointly considered before a true estimate can be made.

Saline Constituents.—The total saline bodies in all waters depend upon the strata receiving the rainfall and through which the rain percolates.

Of the saline bodies which are more or less soluble in water, and which are therefore taken up by the rain water in percolating through the various porous strata, mention may be made to calcic carbonate, calcic sulphate and calcic magnesic and sodic chloride, familiar to us in the mountain limestones of Derbyshire and the marls of Cheshire and Staffordshire.

The great masses of mountain limestone and dolomite or magnesian limestone have a very important influence upon the

waters passing over and through them. The carbonic acid taken into solution by the rain water in falling through the atmosphere and from the surface soil enables this water to dissolve these rocks and take into solution carbonates of calcium and magnesium and small quantities of carbonates of iron and manganese. Other acids, occurring in small quantities in rain water, also act on these rocks and pass into the ground in solution as salts of calcium or magnesium, etc. Chlorides, sulphates, and nitrates of alkalies—sodium and potassium—may be taken into solution, and almost invariably small quantities of silica and alumina. Iron pyrites by oxidation and decomposition supplies sulphuric acid which, reacting with the carbonates, yield sulphates even when gypsum is not found in the soil.

Dr Parkes[1] classifies waters according to their hardness as follows:—

1. **Waters from Granitic Metamorphic Trap-rock and Clay Slate.**—The total solids usually very low, not exceeding 6 grains per gallon; they consist of carbonate and chloride of sodium, with very little lime and magnesia. The quantity of organic matter small.

2. **Millstone Grit and Hard Oölite Waters.**—These resemble the waters just described; they are very pure, and contain carbonate and sulphate of lime, magnesia, and traces of iron; the solids seldom exceed 8 grains per gallon.

3. **Soft Sand-rock Waters.**—Waters derived from this source are frequently impure, and contain much chloride, sulphate, and carbonate of sodium, with but little lime and magnesia. The total solids range from 30 to 80 grains per gallon, and the organic matter is also very high. Occasionally these waters are found pure and soft.

4. **Loose Sand and Gravel Waters.**—Composition extremely variable; solids ranging from 4 to 70 grains per gallon; reaction of water often strongly alkaline, and the organic matter somewhat high. A few are very pure, notably those from the greensand, where extreme purity is sometimes found.

5. **Lias Clay Waters.**—Frequently contain very large quantities of mineral matter; as much, even, as 200 grains per gallon.

6. **Chalk Waters.**—These, representing a very large and most useful class, are usually of fair, and sometimes of great, purity, and contain from 5 to 25 grains per gallon of carbonate of lime.

7. **Limestone and Magnesian Limestone.**—These resemble in many respects chalk waters, but contain large quantities of

[1] *Practical Hygiene.*

magnesia and lime, which exist generally as sulphate. Sometimes they are of great purity.

8. **Senelic Waters.**—Rich in sulphate of lime, and generally very hard, but often of great purity.

9. **Surface and Subsoil Waters.**—These of course vary enormously in composition, but are mostly impure, almost invariably so in populated districts.

10. **Marsh and Moor Waters.**—These are often extremely soft, many moor waters containing a solid residue not exceeding 5 grains per gallon.

Marsh waters are very rich in organic matter; but this is of little importance, as it is usually of a vegetable character.

Moor waters are very similar, but contain less organic matter, are indeed sometimes very pure. They generally have a peculiar earthy or peaty smell, and are of a slightly yellowish tint.

The saline bodies in ordinary waters vary from about 2 to 80 or even 250 grains per gallon, and waters are distinguished as hard and soft according as they contain large or small quantities of lime or magnesia salts in solution. These may exist either as carbonates held in solution by carbonic acid, or as sulphates. In both cases the water is hard: that is, it requires much soap to be used in order to make a lather, because an insoluble compound is formed by the union of the lime or magnesia with the fatty acid of the soap. The hardness, however, is of two distinct kinds, namely, "temporary" and "permanent."

Temporary hardness is due to the solution of carbonates of lime and magnesia which may be precipitated on boiling; whilst *permanent hardness* is due to the presence of unprecipitable bodies, namely, the sulphates of those bases.

Let us now look at the difference in the character and quantity of the saline constituents in what is known to the brewer as hard and soft waters.

The following is a typical analysis of water drawn from the new red sandstone (Keuper) at Burton-on-Trent.

1. Burton Water (Hard).

Calcic sulphate	77·89
Calcic carbonate	6·80
Magnesic carbonate	20·29
Sodic chloride	2·60
Potassic sulphate	1·73
Sodic nitrate	1·32
Total saline bodies	110·63 grains per gallon.

In this and similar waters the calcic sulphate is the chief constituent, the best effect for pale-ale production being provided with waters containing from 50 to 75 grains per gallon. It will be noted that the chlorides are very small in quantity, and although the magnesic carbonate is very high, yet it would be almost entirely precipitated on boiling.

2. Dublin Water (Soft).

Calcic sulphate	4·40	
Calcic carbonate	14·30	
Magnesic carbonate	1·18	
Sodic chloride	1·73	
Iron oxide and alumina	1·14	
Silica	·12	
Total saline bodies	22·87	grains per gallon.

This and similar waters are characteristic for the small amount of saline bodies, excepting calcic carbonate, which they contain. Even this salt, as well as magnesic carbonate, is almost entirely precipitated on boiling, so that practically the amount of saline bodies which enter the mash total about six grains per gallon. Such waters are renowned for black-beer production.

3. Medium Waters.

Calcic sulphate	6·25	
Calcic carbonate	16·24	
Magnesic carbonate	4·14	
Sodic chloride	35·12	
Calcic chloride	3·88	
Iron oxide and alumina	0·22	
Silica	0·26	
Total saline bodies	66·11	grains per gallon.

The characteristic property of these waters is the large quantity of chlorides which they contain, and which renders them particularly suitable for mild-ale production.

Other typical waters rich in chlorides are the following:—

4. Edinburgh Waters.

Calcic and magnesic carbonates	19·86	33·74	
Calcic and magnesic sulphates	6·59	9·76	
Sodic and potassic chlorides	14·57	18·34	
Magnesic chloride	...	2·13	
Total saline bodies	41·02	63·97	grains per gallon.

The carbonates of these waters are also almost entirely precipitated on boiling; the chlorides, which like the former water (No. 3) are excessive, are undoubtedly of advantage in giving the decidedly full flavour to all mild beers produced by their aid.

5. Chalk Waters.

Calcic and magnesic carbonate .	13·60
Calcic sulphate	1·23
Calcic chloride	1·32
Magnesic nitrate . . .	·41
Total saline bodies .	16·56 grains per gallon.

The above is an example of pure chalk waters such as is obtained from the chalky strata of the south of England and numerous other districts.

6. London Waters.

(Derived from the greensand below the London clay.)

Calcic carbonate . . .	6·83
Magnesic carbonate . . .	1·73
Sodic chloride	10·89
Sodic sulphate	14·36
Sodic carbonate . . .	10·31
Silica and alumina . . .	·36
Iron oxide	·48
Total saline bodies .	44·96 grains per gallon.

From these waters the black beers for which London has so long been celebrated are brewed. The sodic chloride, which is in considerable proportion, and also the presence of sodic sulphate, will be noted. Such waters are unsatisfactory for pale-ale production, and it is impossible to artificially manipulate them for this purpose, since after such treatment the chlorides are excessive.

It will be seen, then, that water No. 1 is excellent for pale-ale production, but would require to be artificially treated for the production of mild ale or stout; that waters Nos. 2 and 6 are excellent for stout, but would require to be artificially treated for the production of pale and mild ales; that waters Nos. 3 and 4 are excellent for mild ales, but would require manipulating when producing pale ales or stout; that water No. 5 requires artificial manipulation for the production of pale

and mild ales, but without treatment is a good water for stout brewing; whilst water No. 6 is unsuitable for pale ale, artificial treatment being of no avail.

All the foregoing waters, excepting Nos. 1 and 6, readily lend themselves to artificial treatment, so that any kind of pale, mild, or black beer may be produced.

Artificial Saline Manipulation. — From what has gone before and from general knowledge of the facts:—

(1) That the finest pale ales produced in this kingdom are those manufactured from hard waters such as are found at Burton;

(2) That the finest black beers are produced by the aid of extremely soft waters such as are found at Dublin;

(3) That the finest mild ales are obtained by the use of intermediate waters containing an excess of chlorides, which are common in many parts of the kingdom;

it follows, almost without saying, that as no firm of brewers possesses the desired respective waters, it becomes necessary to artificially treat the supply for one or other or all beers.

In copying the saline character of any special water, we have therefore to add the salts in which our supply is deficient, and sometimes decompose salts which naturally exist; or, if our supply is excessively saline, first dilute it before carrying out either.

An excessively saline water is therefore either diluted by the addition of a pure supply, such as that employed for domestic purposes, or it is boiled for a considerable time by blowing in naked steam.

All good brewing waters should contain at least 6 grains per gallon of calcic carbonate. There is no proof that there is any advantage in the presence of more, but it is a fact that some of the best brewing waters contain a far larger proportion.

If, in the production of bitter beers, it is desired to harden the water, assuming it contains an excessive quantity of earthy carbonates, a portion of them may be converted into sulphites by the addition of sulphurous acid, thus:—

$$H_2SO_3 + CaCO_3 = CaSO_3 + OH_2 + CO_2.$$
$$H_2SO_3 + MgCO_3 = MgSO_3 + OH_2 + CO_2.$$

On the other hand, if such carbonates exist in the liquor in sufficient quantity, the water may be hardened by the addition of sulphate of lime or, what is practically the same, gypsum.

The most suitable amount of sulphate of lime in a water for

"stock" pale ales is 75 grains per gallon, but for running bitters required to drop brilliant in quick time 30 grains is more preferable. In no instance should the former amount be overstepped, since, if so, beers will be slow in coming into condition and in dropping bright.

With regard to sulphate of magnesia, practical experience has shown that the quantity should not exceed one-third that of the sulphate of lime present. If present in large excess, it promotes a tendency to fretfulness and results in thin drinking beers.

Taking the chlorides as next in order, their influence in imparting fulness to beers has long been known; and it is now generally acknowledged that chlorides of calcium and magnesium alone, or more particularly in conjunction with those of sodium and potassium, determine a full and rich flavour. A proportion of chlorides which might be expected to give marked results would be about 7 or 8 grains of calcic chloride, the same quantity of magnesic chloride, 10 grains of potassic chloride, and 8 of sodic chloride per gallon.

The sulphates and carbonates of the alkalies, if present in excess in a brewing water, are decidedly objectionable, even for stout or porter production, for which they were once thought desirable. They extract colouring matter from the materials employed during mashing, and also a rank, bitter flavour from the hops during boiling. They also act upon the nitrogenous bodies by reason of their tendency to neutralise the acidity of the wort, and in consequence prevent any peptonising influence. In order to decompose such objectionable bodies, treatment with chloride of calcium is resorted to, thus:—

$$\begin{aligned} CaCl_2 + Na_2SO_4 &= CaSO_4 + 2\ NaCl. \\ CaCl_2 + K_2SO_4 &= CaSO_4 + 2\ KCl. \\ CaCl_2 + Na_2CO_3 &= CaCO_3 + 2\ NaCl. \\ CaCl_2 + K_2CO_3 &= CaCO_3 + 2\ KCl. \end{aligned}$$

So that in each case useful salts are produced from the injurious ones naturally present.

From the first example we see that, by the addition of chloride of calcium to a water containing sulphate of soda, we get a reaction resulting in their decomposition and the formation of two fresh compounds. It should be borne in mind, however, that by the use of chloride of calcium to decompose sulphates and carbonates of soda or potash, the amount of resulting chlorides is usually so large that, as previously stated, a high-class pale ale can seldom be produced.

The commercial salts usually contain varying percentages of moisture, so that allowance must be made in this direction when artificially treating water.

The following is a list of the salts usually employed in water manipulation, showing their quantities in the anhydrous form; also the percentage of moisture and actual salt:—

Commercial Salt.		Anhydrous Salt.	Moisture Percentage.	Percentage Actual Salt.
Calcic sulphate (gypsum)	$CaSO_4 + 2\ OH_2 = 172$	136	20·93	79·07
Calcic chloride .	$CaCl_2 + 6\ OH_2 = 219$	111	49·31	80·69
Magnesic sulphate (Epsom salt)	$MgSO_4 + 7\ OH_2 = 246$	120	51·21	48·79
Magnesic chloride	$MgCl_2 + 6\ OH_2 = 203$	95	53·20	46·80
Sodic carbonate .	$Na_2CO_3 + 10\ OH_2 = 286$	106	62·93	37·07
Potassic ,, .	$K_2CO_3 + 2\ OH_2 = 174$	138	20·68	79·32
Sodic chloride (common salt)	$NaCl = 58·5$			
Calcic carbonate .	$CaCO_3 = 100$			

An example of the above being:—

$$CaSO_4 + 2\ OH_2$$

$$\begin{array}{ll} Ca = 40 & + O = 32 \\ S = 32 & H = 4 \\ O = 64 & \\ \hline 136 & 36 \end{array}$$

172 : 36 :: 100 : 20·93 per cent. moisture.

100·00 − 20·93 = 79·07 per cent. actual salt. $CaSO_4$.

In cases where it is first necessary to decompose the salts already existing in the water, facts similar to the following have to be taken into consideration:—

From the foregoing table we see that the equivalent of anhydrous calcic sulphate is 136; by dividing this figure into the weight of any commercial salt with which it will combine, we arrive at the quantity of the salt necessary to be added to the water in order to effect decomposition of the existing salt, viz., calcic sulphate in a water = 136, which divided by sodic carbonate commercial 286 = 2·10 grains per gallon. In other words, every

grain of calcic sulphate in the water will require for its decomposition 2·10 grains of sodic carbonate. Similarly—

Calcic sulphate 136 ÷ potassic carbonate 174 = 1·28.
Magnesic sulphate 120 ÷ sodic carbonate 286 = 2·37.
Potassic carbonate 174 ÷ magnesic sulphate 120 = 1·45.

The following table shows the reactions and the quantities of salts required to effect decomposition :—

Every Grain per gallon of		*				
$CaSO_4$	requires	2·10	grains	Na_2CO_3	to produce	$Na_2SO_4 + CaCO_3$.
$CaSO_4$	,,	1·28	,,	K_2CO_3	,,	$K_2SO_4 + CaCO_3$.
$MgSO_4$	,,	2·37	,,	Na_2CO_3	,,	$Na_2SO_4 + MgCO_3$.
$MgSO_4$	,,	1·45	,,	K_2CO_3	,,	$K_2SO_4 + MgCO_3$.
Na_2SO_4	,,	1·20	,,	$CaCl_2$	,,	$CaSO_4 + 2\ NaCl$.
K_2SO_4	,,	1·50	,,	$CaCl_2$	,,	$CaSO_4 + 2\ KCl$.
K_2CO_3	,,	1·20	,,	$CaCl_2$	,,	$CaCO_3 + 2\ KCl$.
Na_2CO_3	,,	1·00	grain	$CaCl_2$	,,	$CaCO_3 + 2\ NaCl$.

All that is necessary is to multiply the number of grains per gallon of each of the salts in a water by the corresponding figures given in the column marked *, and the result will be the total amount (grains) of the respective anhydrous salts to be added to each gallon of the water in order to effect a mutual exchange between the acids and bases. If, then, by these means we completely change the character of a water, knowing the amounts and character of the salts existing after treatment, we may then, if necessary, add any further salts desired.

Owing to calcic chloride being a very deliquescent salt, and in consequence somewhat difficult to handle, it is often purchased in the form of a saturated solution, which has a specific gravity of about 1380.

A specific gravity equal to 1379·7 equals 38 per cent. of calcic chloride ($CaCl_2$) in solution. This figure is per cent. by weight; so that to get per cent. weight on volume—in order that the solution can be measured instead of weighed—it becomes necessary to multiply 38 by the gravity divided by 1000, *i.e.* $38 \times 1{\cdot}3797 =$ 52·4 per cent. Hence a pint of this solution containing 20 fluid ounces contains 52·4 per cent. of 20, or just over 10, ounces by weight of calcic chloride. Therefore a twentieth of a pint to a barrel equals practically half an ounce, or, to be exact, 2·29 grains, equivalent to just over 6 grains per gallon of calcic chloride. In like manner the grains per gallon it required to communicate

to a water can be worked out upon knowing the specific gravity of the solution.

Biological Examination of Water. — Water is very absorbent; whether it be the water of rivers, lakes, ponds, or that in public reservoirs, it readily absorbs gases and receives the floating particles of the atmosphere, commonly called "dust," but which constitute for the most part "germs," which in many cases only require moisture to bring out their vitality.

No kind of water, whether running or standing, is devoid of bacteria, and the waters of rivers usually contain many more organisms than that of lakes or large reservoirs. Some species of bacteria are confined to foul or contaminated waters containing decaying animal and vegetable matter only, such as pools on moorlands, dirty ditches, canals, docks, etc.; and from this it is inferred that it is not only possible, but that it will help us in time to come, when our knowledge of bacteriology becomes more extended, to connect particular forms with particular kinds of water and with particular sources of contamination.

In some cases it is found that there are forms of bacteria in a water which are not typically characteristic of water at all, but are pathogenic species, which have been introduced with foreign matter; these cases are, however, rare.

As the rain water percolates through the strata, more or less of it soon sinks to levels below those at which there is danger of it emerging as a contaminated fluid, and in this process of percolation other important changes take place besides those already enumerated.

As the water passes from the surface soil to the subsoil, it is to a great extent deprived of its dissolved oxygen, and a large portion of its suspended germs are held back in the capillary interspaces. In the subsoil the water is in contact with bacteria of quite a different nature from those in the well-aerated surface soils rich in organic and other food supplies. These anaërobic organisms of the deeper layers are not necessarily less injurious or otherwise than the aërobic forms in the surface soil, but far too little is known about them to say much as to the comparison. Water examined at the source of deep springs, or in the deep subterranean layers tapped by artesian well-pipes, is found to be wholly or practically free from organisms at or near the surface. Moreover, it is an axiom that, in cases where the water supply is drawn from rivers, there are more bacteria as we go towards the mouth, and fewer as we ascend the heights of the watershed; whilst the gain in bacteria, both as regards forms and numbers

of individuals, is marked below each town or inhabited area through which the river flows.

It is obvious that the process of water examination, described in Part IX., enables us to investigate the subject further than is possible by purely chemical analysis, as described in Part VI. By determining the number of micro-organisms present in water before any system of purification, we are enabled to see how far it is contaminated by such organisms; whilst if we subject water to filtration, we are not only thus in a position to test the efficiency of our filters, but to keep a watch on the same, any undue proportion of micro-organisms making their appearance in the filtered water proving at once that the filters are not acting, and that either the filtering material requires to be renewed or cleansed.

According to Schwacktiöfer, who bases his opinion on a large number of analyses of brewing waters conducted by the meat-gelatine plate-cultivation process (p. 369), a water may be classed as good so long as it does not contain more than 6120 colonies per c.c.; unfavourable from 6120 up to 8456 per c.c.; permissible from 8456 up to 46,700 per c.c.; and beyond this bad. This of course does not consider the question of contamination from pathogenic organisms, the appearance of even one such germ being more than sufficient to condemn the supply.

According to Hansen, a water is held to be a satisfactory one if, when examined by his method (p. 372), the wort to which it has been added remains clear after three days' exposure to a temperature of 90° F. (32·2° C.).

In the author's opinion Hansen's method is an excellent one for testing aërial organisms, that is to say, for testing the number of organisms present on any particular occasion in the atmosphere capable of developing in wort, yeast, or beer; but his statement, "*The vast amount of organisms found in water have no significance whatever for the brewer, as very few are capable of vegetating in either wort or beer*," must be accepted with great reservation. We know perfectly well, as the author was the first to show,[1] that absolute sterilisation of wort is effected during boiling in the copper, and that the significance of organisms is their growth in wort after boiling, and in yeast and beer; but we are aware that even such organisms as have been destroyed by sterilisation, or that do not grow in wort, yeast, or beer, and thus suffer destruction, are, though dead, left in the wort, yeast, or beer, and act as food or toxins for other organisms. In any case, therefore, it is, in the author's opinion, advisable to remove them

[1] *Brewing Trade Review*, 1st July 1889, 228.

from a water by filtration, provided they are present in great numbers.

Finally, a water unduly charged with micro-organisms cannot flow into a brewery or malt-house and be used for even cleansing purposes without sowing seeds for bacterial growths in every direction.

Sulphites.

The sulphites and "bi" or acid sulphites offered to the brewer are usually of great purity and strength, and no longer the inferior varieties which were not uncommonly manufactured and met with some fifteen or twenty years ago. The oldest and perhaps still the best of these is bisulphite of lime, and although this has to some extent been discarded for the more conveniently handled solid sulphites, it is nevertheless believed by many to be superior, and certainly has advantages in the sense that it is a valuable antiseptic for washing fermenting vessels and other brewery plant, as well as acting as a germicide when added to beer or to the sprinkling water used during malting.

It is usually sent out at a specific gravity of 1065°, but may of course be obtained at greater strength, such as 1075°, or even up to 1140°. If of a higher specific gravity than 1065°, it rapidly loses its strength on storage owing to the extreme liability of sulphur dioxide to volatilise, so that it is questionable whether it is not more advisable to purchase at the lower strength. In any case, the lower strength is sufficient for all brewery purposes. Bisulphite of a strength of 1065° should contain not less than 6 per cent. total sulphurous acid, one-half of which should exist in the free state. Most samples contain sulphate of lime, but in carefully prepared samples the amount of this should be less than 0·5 per cent. Traces of magnesia and chlorides of the alkalies are usually present, but exert no deleterious influence. Any sample showing the presence of hyposulphites should be discarded as unfit for use.

Sulphurous acid is manufactured by heating coke which has been moistened with sulphuric acid, or by boiling sulphuric acid in the presence of sulphur in a boiler similar to a steam boiler. The sulphurous fumes evolved are then passed through a washing apparatus, where they are purified and condensed.

In the manufacture of bisulphite of lime the purified and condensed acid is conducted into a series of lead-lined tanks, or casks containing milk of lime.

Sulphite, and afterwards bisulphite, of lime is thus produced.

Sulphites of soda and potash are also largely used, but are very prone to decompose during storage, sulphate of soda on the one hand, and sulphate of potash on the other, being formed.

Besides the sulphites and the bisulphites, a series of salts have long been prepared which are known as the "metabisulphites," also sometimes called pyrosulphites and disulphites. Thus we have potassium metabisulphite, kalium metasulphite, etc.

Kalium metasulphite is prepared by passing sulphur dioxide into a hot saturated solution of potassium carbonate (Muspratt), or into a mixture of milk of lime and potassium sulphite (Boake Roberts).

The crystals with difficulty dissolve in water, and have an acrid, unpleasant taste. Many years ago Boake Roberts obtained a patent for mixing the crystals with a gummy substance and compressing certain quantities into "tabloids"; the patent, however, has expired, and the word "tabloid" is now the exclusive property of Burroughs Wellcome & Co.

The value of the sulphites, bisulphites, and metasulphites to the brewer depends upon their decomposition by acids. When the beer to which these materials are added develops acidity, the sulphites are decomposed, the liberated sulphurous acid effecting the destruction or oxidation of the organic matter and thus preventing putrefaction. In other words, any disease organisms which make their appearance (lactic, butyric, etc.) are oxidised and destroyed before they have time to multiply.

Barley and Malt.

Barley.

Weight.—The weight of a good two-rowed barley varies from 49 to 58 lbs. per bushel, the best averaging from 53 to 58 lbs.; while six-rowed bere or big, having a thick husk, usually weighs from 49 to 51 lbs. per bushel; and foreign varieties proportionately less in comparison to their greater proportion of husk. Much importance was formerly attached to the weight, but provided the same essential characters are present, that is to say, provided the grain is not damp, that it is comparatively free from extraneous bodies, that the corns are of equal size and colour, that there are no dead, sprouted, or weathered corns, and that the odour is good, light barley will yield equally good malt. This is especially the case with the foreign barleys, although it must be remembered that the starch content is less in foreign grain; and as the

extract obtainable from barley, or the malt made from it, depends upon its quantity of starch, this must be reckoned with when purchasing.

Starch.—As a rule barley contains from 60 to 66 per cent. of its weight of starch.

Proteids or Albuminoids.—Steely barleys generally have a high nitrogen content, or, in other words, possess a large percentage of proteids or albuminoids. Barley with a moisture content of 15 per cent. (which may be taken as an average) contains from 1 to 2·5 per cent. of nitrogen, equal to from 6 to 16 per cent. albuminoids.

An excess of nitrogenous matter displaces more or less soluble or hydrolysable carbohydrate matter of the endosperm. There is therefore loss of extract, because the greater part of the nitrogenous matter is not available as extract.

Barleys of high nitrogen content, say from 1·8 per cent. upwards (=11 per cent. albuminoids), are generally difficult to modify unless forced, which means loss of material by undue respiration. The difference in amount of extract, calculated on the barley, even if there were no loss due to increased respiration or imperfect modification, may be considerable. Take extreme cases, with a barley containing 2 per cent. of nitrogen (12 per cent. albuminoids): for every 100 lbs. of barley there will be, roughly, 12 per cent. of nitrogenous matter, of which not more than 2½ per cent. will be found in the boiled extract from the malt; the remainder, 9½ lbs. or more, will be found partly in the rootlets of the malt, partly in the spent grains, and partly in the matter separated on boiling. On the other hand, with grain containing only 1 per cent. of nitrogen, equal to 6 lbs. of nitrogenous matter per 100 lbs. of barley, about 1½ lbs. of nitrogenous matter may be found in the malt extract after boiling, and only 4½ lbs. lost.

It is to be understood that these are extreme cases, neither as low a nitrogen percentage as 1 nor as high as 2 (6 to 12 per cent. albuminoids) being very common. But, on the other hand, barleys with 1 and 2 per cent. respectively of nitrogen will never be alike in other respects. The 1 per cent. barley may be a very thin, husky grain, and the low nitrogen content be due to excess of husk, which contains less than half as much nitrogenous matter as does an equal weight of endosperm; whilst grain of good size, but very thin husk—such as a fine sample of Chevallier Chilian—may have a relatively high nitrogen content.

We thus see that 80 to 85 per cent. of the nitrogenous matter in barley is insoluble; but nitrogen content alone is only useful as an index of quality for comparing barleys which are in other respects approximately alike, and all we can say is that barleys of high nitrogen content will necessarily, when converted into malt, give a lower extract to the extent that proteid matter displaces available carbohydrate matter, and that also nearly always the barley will be steely, and require to be so malted as to cause undue rootlet growth and undue loss by respiration.

In the author's opinion barley should not contain more than 11 per cent. of nitrogenous substances, or forcing during malting becomes necessary.

Acidity.—An aqueous extract of barley is always more or less acid; and this, like the acidity of malt, was formerly attributed to the presence of lactic acid. It is now universally agreed, however, that the acidity is mainly due to primary and acid phosphates, and in a less degree to volatile and fixed organic acids.

The respective proportions of these bodies in two samples of barley, expressed in percentages of lactic acid on the water-free substance, are given by Prior as follows:—

	1	2
Volatile organic acids	0·07	0·05
Fixed organic acids	0·06	0·05
Primary phosphates	0·29	0·25
	0·42	0·35

It has not yet been proved that an excess of acidity is detrimental or otherwise, and the test, so far as barley is concerned, is of little use.

Mineral Matters.—On incineration, barley leaves a residue or ash of from 2·5 to 3·0 per cent., reckoned on the dry substance. This ash, expressed as percentage on the ash, consists approximately of the following mineral matters:—

Potassium	20·92
Sodium	2·39
Lime	2·64
Magnesia	8·83
Iron oxide	1·19
Phosphoric acid	35·10
Sulphuric anhydride	1·80
Silica	25·91
Chlorine	1·02

The potassium and magnesium phosphates are the most important, since they supply the mineral food for the yeast.

Moisture.—The average amount of moisture in British barleys in good harvest seasons varies from 12 to 18 per cent., and in wet seasons may run as high as 25 per cent.; the average, however, may be taken as 15 per cent., and barley containing over 20 per cent. may be considered damp.

With foreign barleys the moisture is frequently as low as 7 and seldom more than 12 per cent.

Physical Examination.—There can be no doubt that the chemical examination of barley, as described in Part VI., is of considerable importance; and although, from a botanical point of view, a great deal of work has been performed and much light thrown upon the cereal, nevertheless it must be admitted that the analyst has fought shy of examining it. The time is now ripe, however, for a more complete chemical analysis than we at present possess, and in the near future more will be known of the character of barley and its use in malting and brewing. We know that the translocation diastase varies approximately *pari passu* with the total nitrogen with barleys grown under parallel conditions, and that the enzyme forms products by its action on starch paste which are quite different from those produced by malt diastase. We have also ample evidence that differences are exhibited in the starch of different barleys, and are no doubt explicable by the conditions of growth, harvesting, and the ripeness of the grain. We can only as yet, however, surmise that these differences are due to the latter conditions; and from these facts it behoves chemists to attack these questions with vigour, and bring to light more facts regarding the constituents of barley and their action during the growth of the grain during malting and as existing in finished malt. Barley when offered for sale to the brewer or maltster has always been threshed from the straw and separated from the awns, and in judging the quality the following points have more or less to be carefully considered :—

(1) Mould: whether derived from field, stack, or granary.

(2) Odour.

(3) Bittenness: the ravages of insects or vermin.

(4) Dirtiness: occasioned by the presence of dust, dirt, stones, string, metal, or other so-termed rubbish.

(5) Threshing influences: brokenness, the damage arising from threshing, hummelling, or dressing upon germ or kernel. The presence or absence of awns: if present, to what extent; if absent, to any detrimental closeness.

(6) Condition of endosperm: mealiness, softness, colour as distinct from colour of skin, steeliness.

(7) Size: uniformity.

(8) Colour: uniformity.

(9) Moisture.

(10) The influences derived from weathering or imperfect sweating in stack.

It is not within the range of the present work to deal with these matters *in extenso*, but rather with the points of analysis, so we must leave these subjects, sufficing it to say that it must be obvious to all that highly important and absolutely essential as is a true evaluation of barley from a physical examination, this should be supplemented in very many instances by chemical analysis, the question of nitrogen content alone being sufficient to guide one as to the method of procedure during malting.

MALT.

Weight.—The weight of pale malt from British barley varies from 38 to 44 lbs. per bushel, and more highly dried and foreign varieties proportionately less. The heavier the malt, provided it possesses the other necessary qualities, the more the extract obtainable. Care should be exercised, however, to see that the malt does not derive this quality from imperfectly vegetated or steely corns, which are naturally heavier than those of a friable nature.

Extract.—The laboratory extract of malt depends largely upon the system of grinding—whether coarse or ground to powder. By means of the Seck mill (fig. 70, p. 196) a standard system may be employed and comparisons the better understood. By grinding in the Seck mill set at 25°, any average pale British malt gives a laboratory extract of from 93 to 96 lbs. per standard quarter. Mild ale malts should show from 90 to 94 lbs., and foreign grain according to its character. Fine samples of Californian in exceptional cases may show up to 92 lbs., but 86 to 90 is more usual. Smyrnas' are a little lower; Tripolis', Tunisians' and the like from 83 to 85; and Ouchacks' from 83 to 91, the former being more usual owing to the difficulty of getting such material thoroughly modified.

Saccharification Period.—It is understood by this test that the shorter the period taken for the starch to disappear, the better the modification of the malt. It is a test of some importance, in so far as it shows that in cases where the diastatic power

is found to be high, the saccharification of the malt is accomplished in quicker time, and hence the saccharification period and the diastatic power have some connection. There is, however, a dislike for any quantitative method which depends essentially upon a starch test when there is much carbohydrate matter present which is similar to starch, and on the whole the significance of the test lies only in the corroboration it affords the more exact and important test for diastatic power as devised by Lintner.

Specific Rotatory Power of Wort.—The value of this test is best explained in the words of Heron, who states—

"In my opinion the specific rotatory power of the hot mash is a very useful factor in the examination of malt, as giving some idea of the relative ratios of dextrin to maltose. I find that a wort which gives a specific rotatory power of $[\alpha]_D = 116$ shows a dextrin maltose ratio of about 1 : 3, and these are generally the conditions which govern the brewing of pale and stock ales, whilst for mild and running ales a specific rotatory power of $[\alpha]_D = 110$ is usually found to be advisable.

"Mashing with the same malt and under the same conditions, the brewer will find that the same polarimetric results are obtained day after day. From this it will be seen how extremely advisable it is to make a polarimetric examination of each lot of fresh malt in the laboratory before proceeding to use it on the large scale in the brewery, for if the brewer when examining his wort obtains a lower $[\alpha]_D$ than usual, it serves as an indication that something must be done to restore it to the normal, either by raising his striking heat or blending this malt with another which gives a higher $[\alpha]_D$ than the usual, and thereby bring about a normal condition of things; otherwise variations in attenuation and trouble in fermentation and cask conditioning may arise.

"It is usual nowadays not to brew from one single class of malt, but to use a blend of two or more different kinds, generally English, with one or two foreign varieties. It will be found extremely useful to make a preliminary mash on the small scale of each of such malts, and determine the $[\alpha]_D$ of each before using them in the brewery."

In order to make this test of value, it is absolutely essential that the temperature of the mash throughout should be the same with each class of malt operated upon, since slight fluctuations of temperature cause considerable difference in the specific rotatory power. The same remarks apply with even greater force to the cupric oxide reducing power, so that both these, whether expressed

as maltose, dextrin ratio, or otherwise, are only of value when the conditions of temperature are constant.

Acidity.—Formerly the acidity of malt was attributed to the presence of lactic acid, but it is now quite a moot point whether lactic acid exists in normal malt, it being almost universally agreed that the acidity of malt is due, as Prior has shown,[1] to primary or acid phosphates, and in a less degree to volatile and fixed organic acids.

During the early period of the germination of barley the volatile acids remain about the same, or may increase somewhat, whilst the fixed organic acids suffer a perceptible diminution; this diminution is concurrent with a proportionate increase of primary phosphates, the explanation being that the fixed organic acids react with the secondary and tertiary phosphates of potassium, magnesium, and calcium, giving rise to primary phosphates and salts of the organic acids.

The normal proportion of free acid in malt is from 0·2 to 0·3 per cent., expressed in terms of lactic acid, and it should not exceed 0·4 per cent.

Although a high acidity in malt is suspicious, many of the worst malts possess but little. High-kilning temperatures tend to increase acidity, the acid arising from the destruction or caramelisation of organic matter. A badly stored malt always shows a high moisture percentage, and commonly with this a high acidity. It must be remembered, however, that malt just off the kiln may, from imperfect kilning, contain an excess of moisture, and the acidity may be low.

Although the method of expressing the acidity in terms of lactic acid is purely conventional, it nevertheless suffices for purposes of comparison. The indicator employed in titrating with $\frac{N}{10}$ sodic hydrate is important, since it has been shown that phenol-phthalein gives values at least four or five times as high as rosolic acid or litmus, both the latter giving practically the same results. In the author's opinion it is preferable to employ litmus paper.

Matters soluble in Cold Water and Ready-formed Soluble Carbohydrates.—As diastase is able to act upon starch in the cold, it naturally follows that its presence will be attended with the products of its action on the starch of the endosperm of barley during germination.

During germination a certain quantity of the constituents of

[1] *Chem. und Phys. des Malzes und des Bieres*, 40.

the endosperm is rendered soluble and transformed into food for the growing plant. It is unnecessary to enter into details as to the manner in which the food is elaborated, but from the amount of the bodies thus rendered soluble and contained in the finished malt, much information is gained as to the character of the malt and method of its manufacture.

The quantity of soluble carbohydrates formed is influenced by the mode of treatment of the barley during vegetation on the floor and during kilning. Moritz and Morris consider that an excess of ready-formed sugar, or better expressed as ready-formed soluble carbohydrates, in malt, point to errors in the malting process. One or two things has happened: either the malt has been forced, that is to say, its growth has been hurried at either too high a temperature or with too much water, or the green malt has been loaded to kiln too fresh, that is, containing too much moisture.

In the former case an excess of soluble matters is produced; in the latter case the food which should have been normally assimilated by the root remains in the grain.

In their opinion the ready-formed soluble carbohydrates should not exceed 16 per cent., and malts containing a higher percentage than this are stated to give bad results in brewing.

Abnormally low percentages (under 10 per cent.) point to insufficient germination.

In this way Moritz and Morris propose to use the estimation of ready-formed soluble carbohydrates as a test for the way in which the malting process has been conducted. The utility of the method has, however, on several occasions been contested, and it is evident that an improved method of estimation is required.

The amount of ready-formed soluble carbohydrates should not in themselves be taken as an index of "forcing" or "non-forcing," since it is frequently found that malts showing an excess of these bodies may yet give good results in brewing. A malt may in fact show an excess of these bodies and yet be excellent in other respects, so that it should not be condemned on this factor alone. On the other hand, a "forced" malt always shows a high percentage of ready-formed soluble carbohydrates, and of soluble coagulable albuminoids, and the percentage of acid will also be high in proportion, so that the test when compared with other results is most valuable.

Roughly, we may allow for English pale-ale malt up to 15·5 or 16 per cent., about 16 to 16·5 for mild-ale malt, and up to 17

per cent. for running, black-beer malts. For stock black beers, however, the percentage should not exceed 16. With foreign malts the percentage may be up to 14·5 for Californian, showing an extract of 88 lbs. per quarter, and from 11 to 14 for thinner varieties.

It is not, then, so much the existence of these products as such that constitute the danger, but the fact that their excessive formation usually corresponds to the production of undue proportions of soluble uncoagulable nitrogenous extract. It is difficult to conceive how a difference of even less than 0·5 per cent. in the proportion of uncoagulable nitrogenous extract should influence the stability of brewery produce, but practical experience proves that the continuous use of malts of this character speedily conduces to yeast weakness, the organism apparently becoming supersensitive to modifications in wort composition.

Colour.—The determination of the colour value of malt wort is one over which there has been great controversy. Many analysts record the colour as found by examining the 10 per cent. wort from the miniature mash made for determining the extract value; others are content by recording the colour of a 10 per cent. cold-water extract; others, again, employ either of these worts and calculate the result to percentage on the malt; whilst more generally the colour is established from the hot 10 per cent. mash wort, and then by a rule-of-three sum it is worked out what the colour would be for a gravity of 1055.[1]

Since the colour depth does not vary directly as the density, this latter method is obviously wrong. The method described in Part VI. has been shown by Heron to be correct, and should therefore be adopted. The colour of a malt is undoubtedly a good criterion of the firing during kilning, and, taken in conjunction with the diastatic capacity, is a safe criterion as to whether the malt has received proper treatment during kilning.

A diastatic power for a pale-ale malt ought not to be below 35° or more than 44°, a mild-ale malt from 23° to 30°, and a high-dried malt from 15° to 23°. The tintorial values, in like manner, for a pale-ale malt ought not to be below 4° or more than 6°, a mild-ale malt from 10° to 15°, and a high-dried malt from 15° upwards.

When we find a pale-ale malt possessing a diastatic power of 40° and a tintometer value in the 1 inch cell of 4 units of colour, we know that we are dealing with a malt of good quality. Such a malt will show that the growth on the floor has been carefully

[1] *Briant's Laboratory Text-book*, p. 128.

attended to, that proper withering has taken place, that it has been carefully and thoroughly dried on the kiln before the temperature was raised to any appreciable degree, and that the curing was carried out in a proper and efficient manner.

On the other hand, when we find a malt showing such results as the following: a diastatic power of only 18° to 20° and total units of colour 3—such a malt will be hard to the bite, possess moisture above the normal, and be unsatisfactory in every respect; and although these results do not necessarily imply that the malt has been carelessly grown or improperly attended to whilst on the floor, one thing they do show, and that is, that the malt has been spoilt on the kiln.

In all probability the malt has been loaded to the kiln very imperfectly withered, or not at all, and with a great deal too much moisture in it. In such cases forcing, in the truest sense of the word, takes place in the early stages of drying; and in order to avoid this, and reduce the moisture as quickly as possible, the temperature is speedily raised. By such means the diastase becomes very much restricted, and there is danger if the temperature be carried much higher than 160° F. (71·1° C.), of a large amount of colour being produced, hence insufficient curing must necessarily follow.

With a properly grown, well-dried malt it is possible to raise the curing heat to, and maintain it at, a very much higher temperature than could otherwise be done, without producing any appreciable increase in colour.

Diastatic Power.—The method adopted is based upon the researches of Kjeldahl,[1] and depends upon the amount of reducing sugars produced when extracts of malt are allowed to act upon a solution of soluble starch under defined conditions, the most important of which are that the proportion of diastase employed relative to the starch to be transformed, and the time of the reaction, shall be sufficiently limited to prevent the formation of reducing sugars in excess of a K of 25 as 30, or expressed in the maltose equivalent as R of 40–50. Operating under these conditions at an elevated temperature approximating to 140° F. (60° C.)—a temperature that he had proved as most favourable to the action of the enzyme—he established the law that the relative diastatic power of two malts is proportional to the sugar produced. Some ten years later C. J. Lintner[2] modified this gravimetric process, allowing the action of diastase to take place

[1] *Compt. rend. trav. Carlsberg Lab.*, 1879.

[2] *Wochenschrift für Brauerei,* 1886, 733, 753.

in the cold, and adopting a volumetric method for determining the exact amount of reduction. It is the "Lintner" process that is described in former pages and the one now generally employed; standards, as already stated, ranging from 15° to 23° being regarded as normal for high-dried malts, 23° to 30° for mild-ale malt, and from 35° to 44° for pale-ale material.

Whatever the diastatic power of the malt when loaded to kiln, during drying the diastase is reduced about one-half; but the actual reduction depends upon the moisture content of the green malt when loaded, and the temperatures employed, particularly during the early stages of kilning. Obviously a high-dried malt contains less diastase than a low-dried one, and the two classes of malt require appropriate mashing temperatures—low for those low in diastase, and high for those containing much diastase. Yet mashing temperatures must not be governed alone by diastatic power, but by the tenderness of the malt, a well-made, tender malt permitting the use of a higher mashing temperature than a steely one.

The diastatic power of malt should not be below 15° or over 44°. If below 15°, it points to the malt being inefficiently vegetated; whilst if above 44°, either the diastase has not been properly reduced during kilning, or the malt has originally been abnormally diastatic, which proves faulty manufacture. The colour and diastatic power should have some connection; for instance, the presence in a high-dried malt of a proportion of diastase normal to a pale-dried malt, shows the original diastase to have been excessive.

Proteids or Albuminoids.—A malt contains a slightly less amount of nitrogenous matter than the barley from which it is made, since a portion of the nitrogenous substances passes into the rootlets which are removed from the finished malt. During malting, however, a large proportion of the insoluble nitrogenous bodies present in the barley are rendered soluble.

The average percentage of the total nitrogenous bodies and of the soluble modifications may be taken on English malt as follows:—

Total nitrogenous matter . . .	9·80 per cent.
Soluble ,, ,, ...	2·50 ,,
Insoluble ,, ,, ...	7·30 ,,

With malts sufficiently forced so as gradually to produce actual trouble, the soluble nitrogenous bodies will run up to from 2·8 to 3·2 per cent.

Foreign malts should not show more than 1·8 or 1·9 per cent. for the normal percentage of ready-formed soluble carbohydrates which they contain (11 to 14 per cent.).

Malt made from Danubian barleys, however, show up to 2·4 per cent., and are decidedly unsatisfactory brewing material.

Moisture.—The presence of moisture in malt varies with the conditions of kilning and the method and time of storing. A malt just off the kiln, if properly cured, may contain as low as 0·5 per cent., but shortly afterwards the moisture will be found as high as 1·5 or even 2 per cent.

Malts coming from a distance absorb moisture during transit, and usually contain from 2 to 3 per cent. of moisture.

A malt containing from 3 to 4 per cent. of moisture may be considered "slack," and such malt should be redried before use. Beyond 4 per cent., provided the slackness is due to faulty storage, the malt should be condemned as unfit for use, as it will have suffered internal deteriorations which, though not readily expressed in definite terms, cannot fail to show their bad influence on the flavour and keeping properties of the beer.

Physical Examination.—The chemical examination of malt is admitted by all to be essential to the true evaluation, and the foregoing details all more or less go to prove this fact. It is undoubtedly true that the value of a malt may be well judged by a physical examination, but it is equally true that appearances are at times deceptive, and that although a malt may be good so far as the eye, the nose, and the mouth can detect, it may nevertheless prove bad by chemical analysis. In any case it is impossible, by physical examination, to arrive at the degree of acidity, diastatic power, extract, albuminoids or moisture, and the importance of these factors are obviously known to the practical man. Again, it is impossible, by physical examination, to state whether the malt has been forced or correctly kiln-dried, or whether it is suitable or unsuitable, from a diastatic point of view, for the manufacture of any particular class of beer. On the whole, malt, unlike barley, cannot be judged other than both physically and chemically; and although the former is essential, the latter is equally important, not only where value is concerned commercially, but as to whether or not the malt is capable of answering the particular purpose for which it is intended.

From a physical point of view the "bite" of the malt is important, and instead of a corn here and there being selected and bitten in two, it is best to chew a mouthful, noting the tenderness and flavour and also the colour of the well-chewed

material when removed from the mouth. By these means the experienced man can form an excellent opinion as to the degree of modification, the amount of firing the malt has received, and the general character of the malt from the flavour it yields.

It is impossible to describe the characteristics in these respects which go to define the quality of a malt, and it is only by long practical experience that one can form a correct judgment. They are factors, however, of every importance, and absolutely necessary, in conjunction with a chemical analysis, in the true evaluation of malt.

Again, the degree of growth by examination of the acrospire of several corns, the selection of steely or semi-vitreous corns and the separation of "misses" or dead corns which may be looked upon more or less as raw grain, are also necessary factors in judging the quality of malt; whilst lastly, although neither physically nor chemically is there any test competent to distinguish between "new" and carefully stored matured malt or "new" and redried old grain, the practical man nevertheless fully experiences the influence of age upon brewing results: perhaps, however, the loss of interest on locked-up capital reduces the chance of the otherwise certain appearance of redried old grain.

Biological Examination of Barley and Malt.

The biological examination of barley and malt, if justly judged, is of some considerable importance. It has been shown by the experiments of Becker that barley steeped in the ordinary manner carries with it to the floors a tremendous number of micro-organisms; in fact their numbers, capable of development, vary from 5,500 to as many as 12,350,000 in each gram of the barley, and as every one of these organisms or their spores may, under favourable conditions, multiply in 24 hours to 30,000 or even 300,000, it is self-evident that it is important to wash barley prior to steeping.

Green malt naturally carries an enormous number of micro-organisms, many of which are destroyed by the kilning temperatures; but before the kilning takes place, fermentations of an undesirable nature often set in and result in the communication of objectionable odours and flavours to the malt, causing chemical changes of an undesirable nature to take place and cloudiness in finished beer.

The value of the biological test with malts is important, and centres in the fact that as the acidity they contain is mainly due

to soluble phosphates, the worts they yield nourish bacteria, so that the higher the acidity the larger the quantity of phosphates and the greater the nourishment and the quicker the time in which the wort turns cloudy and putrifies.

In the author's opinion, a wort which remains clear on the forcing tray for 36 hours is particularly sound, whilst one which turns cloudy in a lesser time is unstable.

Colour Malts.

Amber. Crystal. Brown and Black.

Amber malt, like crystal and brown, is employed by brewers chiefly to impart flavour to beers, black malt being used mainly for the purpose of imparting colour.

In the manufacture of amber malt the green malt is taken from the floor at the withering stage, and is loaded on the kiln at a depth of about 4 inches. The fuel used at the early stages of drying is the same as in ordinary malting; but when the malt is hand-dry, the heat is augmented and very dry beech-wood is thrown upon the fire, the products of combustion imparting the desired flavour.

Very high-dried amber malts give a certain viscosity to beers produced by their aid. Thausing states this to be due to the existence of parapeptones in the malt, which give both colour and viscosity.

Crystal, brown, and black malts are manufactured by taking malt at the withering stage, earlier than when manufacturing amber, and roasting in a perforated cylinder enclosed in a cast-iron casing and which can be freely turned.

A low coke fire is employed, and during heating the malt is cautiously and slowly turned.

As the steam passes off, the fire is made up and the heat increased. In 30 minutes a fine rich aroma is evolved from the malt. Having imparted the desired colour and flavour, the malt is then removed from the cylinder and placed on a floor to mellow and cool.

If brown malt is desired, the heating is continued, and in 5 or 10 minutes a good brown or chocolate colour is imparted; whilst if black malt is wanted, the heating is further continued, and in 40 minutes or so the operation for this class of malt is complete.

Brown and black malts are sometimes finished off with a glaze.

This is performed by sprinkling the grain with a sugar solution a few minutes before finishing off: the glaze so imparted darkens the colour of the husk.

In place of sugar solution, glycerine, either alone or with water or steam, is sometimes employed for the same purpose, it being claimed for this mode of treatment that the malt, unlike other varieties, is free from acrid or bitter flavour, and that it possesses a higher colouring power.

Weight.—The weight per bushel of black malt varies a good deal according to the quality; the average medium-priced material weighs about 31 to 32 lbs. per bushel, but very high qualities are frequently as much as 36 and even 37 lbs. per bushel. But these must not be confounded with roasted barleys, which also weigh high.

Brown and crystal malts weigh from 31 to 33 lbs., and amber from 38 to 40 lbs. per bushel.

Extract.—The lbs. per quarter extract obtainable from black malts varies from 50 for low quality up to 70 for high-class material. Brown and crystal malts, under usual conditions of grinding, give from 55 to 60 lbs. in each case. But the fineness of the grinding has a marked effect on the extract with respect to crystal malt especially, and if it be ground as fine as possible, the extract will go as high as 75 lbs. or more. There are somewhat different opinions as to what constitutes an amber malt, some brewers expecting much more severe curing than others; and the extent of the curing affects the extract. As a general average the extract of amber is about 84 to 85 lbs. per standard quarter.

Moisture.—Colour malts are highly hygroscopic or deliquescent, and upon arrival at the brewery will usually be found to contain at least 3 per cent. of moisture, and upon storage for a few weeks the moisture percentage will run up to 5 or even more. It is inadvisable, from this fact alone, to keep any large stock; in fact the majority of brewers generally purchase only in small quantities as and when required.

Colour.—There is no standard method for ascertaining the colour value or tintorial power of these so-called colour malts, but on strictly commercial lines it is perhaps advisable to express colour results in degrees of tint of a solution of 1 lb. gravity in a 1 inch cell examined by Lovibond's tintometer, and compare this with the price of 1 lb. of extract. On the other hand, it is of importance to the brewer to know what colour these malts in definite proportion will add to his worts, and in such instances it

is best to approximately average brewery conditions in obtaining a solution. Hence it is best to mash such proportions of the colour malts as are used in practice with pale material of known tint, and note the increase in colour due to the colour malts. For purposes of comparison, however, a 0·01 per cent. solution, examined by the tintometer as described in Part VI., is valuable.

Physical Examination.—The better the quality of the green malt employed for the manufacture of colour malts, the better the quality of the resulting products.

A sample of roasted malt is uniform in colour, the interior of each corn of a chocolate hue, not an intense black, and each corn clear and clean. If the interior is an intense black with the corns burst, and especially if matted together, it is of a most inferior kind, and neither good flavour nor permanent colour can be obtained from it.

Raw Grain.

Maize Grits.—The oil should be under 1 per cent.; nitrogenous bodies not more than 9 per cent.; starch not less than 74 per cent.; moisture not more than 14 per cent.; and the extract derivable should be from 95 to 100 lbs. per quarter of 336 lbs.

Rice.—As rice contains more starch than maize, and as the starch gelatinises more readily than maize starch, the extract derivable is obviously greater. The extract yield is from 100 to 105 lbs. per 336 lbs. The oil should be under ·8 per cent.; nitrogenous bodies not more than 9 per cent.; starch not less than 79 per cent.; and moisture not more than 14 per cent.

Granulated or flaked Maize and Rice.

	Maize.	Rice.
Starch, not less than	79·0	81·0
Oil, not more than	1·5	0·4
Nitrogenous bodies, not more than .	9·5	8·5
Moisture, not more than . . .	8·0	8·0

Particular care should be taken to observe that the starch of prepared flaked or granulated maize or rice is not steely, as, if so, it not only points to faulty manufacture, but the starch is exceedingly refractory in conversion and the extract obtainable consequently less; for the same reason the extract should not be calculated from the percentage of starch found.

Hops.

A good hop from both the grower's and the brewer's point of view, according to Percival,[1] should have, as far as possible, the following characteristics:—

(*a*) The yield should be large, and the hops should be capable of hanging on the plant without damage for some time, so as to allow a considerable area to be picked and managed with a moderate number of hands.

The time which a hop will remain in good condition without "going off" depends upon the manuring, season, and locality to some extent, but there are constitutional differences among hops in respect of this quality. Fuggle's hops, for example, usually hang well, while the thinner-petalled varieties are easily discoloured and fall in pieces when left a few days in the picking season.

(*b*) The plants should be hardy and highly resistant to the attacks of fungi and aphides.

Unfortunately, delicacy and weakness are almost invariably met with among hops of the best quality.

(*c*) The brewer aims at high lupulin content, for the chief use of the hops to him depends upon the amount of resins present in them. Moreover, for the process of dry-hopping, a pleasant aroma is essential.

So far as the keeping quality of the beer is concerned, and also to a large extent the peculiar bitterness imparted to the liquor by the hops, a sample of good resin content, with only a passable or even poor aroma, is as useful as one of fine aroma, or the hops of the Weald or less-favoured districts would not be grown. However, to impart the most delicate and attractive flavour to beer, only hops of the best quality can be employed. The size of the resin glands, their weight, and the number on each "petal," are generally greatest among hops of poor aroma; but their weight, compared with the rest of the hop, is often higher in the best quality hops than in those of poor aroma and flavour.

In a good brewer's hop the petals should be well covered at the base with lupulin, and the "strig," "petals," and "seeds" should weigh as little as possible.

Hops with the most delicate aroma possess thin, smooth, pale-golden "petals," the bracteoles being well rounded at the tip, and the stipular bracts similar in colour and texture, and broadly oval in shape.

[1] *Jnl. Royal Agric. Soc. of England*, vol. lxii., 1901.

Those of poor aroma have "petals" which are generally rough, thick, and puckered. The bracteoles are more pointed, the stipular bracts being narrow, and often a darker green tint than the bracteoles, so that the colour of the hop is not uniform all over, and cannot be made so, even by the strongest application of sulphur on the kiln.

The worst variety has the small stipular bracts, at the base of the strobile, twisted.

Some varieties of hops have exceptionally pale, straw-coloured "petals" which are very thin; such are always deficient in resin glands, but the aroma is generally good.

A sample of hops, if perfectly dried, is extremely elastic; when released after being pressed, it immediately resumes its former bulk.

Hops which are used for dry-hopping should not disintegrate too readily, or, as it is termed, be "marshy," since the detached fragments float in the beer, and are drawn off from the cask along with it.

Marshiness is either caused by overripeness or some defect in drying.

Lastly, hops should be free from mould, and in no instance should any be accepted which possess the slightest objectionable odour.

The respective proportions of the different constituent parts of the hop cone mechanically separated, are given by Haberland as follows:—

Lupulin	7·92	to	15·70	per cent.
Leaves	69·79	,,	78·36	,,
Stems	8·50	,,	17·54	,,
Ripe seeds . . .	0·02	,,	7·80	,,

Hops are in their prime condition only after from 2 to 3 months' storage. The oils previous to this sometimes tend to cause cloudiness in finished beer, and the practical brewer, as a safeguard, therefore uses considerably greater proportions of "yearlings," as hops twelve months old are called, than of new hops. When two years old they are designated "old hops," and at the end of three years, by which time they are of little or no value, they are termed "old olds."

The case is different, however, with cold-stored hops. Cold storage reduces, but does not entirely arrest, change. For practical purposes, however, cold-stored hops, if of good quality, undergo very little deterioration in the course of one or two years,

and it sounds to sense that beyond this it would, for commercial reasons, be unwise to store them.

Hard and Soft Resins.—The following shows the average percentages of hard and soft resins in new hops:—

New Hops.	Non-preservative Hard Resins.	Preservative Soft Resins.	Total.
Worcester	5·03	7·70	12·73
Sussex	5·40	9·06	14·46
East Kent	3·80	10·74	14·54
Goldings	4·12	11·42	15·54
Californians . . .	8·20	12·46	20·66
Bavarians	8·10	11·42	19·52

Deterioration of Hops as judged by the Oxidation of the Tannin.—

		New Hops. Tannin per cent.	4 Years old. Tannin per cent.	8 Years old. Tannin per cent.
Sussex	1.	6·2	2·2	1·3
	2.	5·4	2·6	1·2
	3.	4·0	0·8	0·6
	4.	2·7	1·2	none
Best Kent	5.	4·6	1·2	none
	6.	3·8	0·9	0·2
	7.	3·3	0·7	none
	8.	4·0	none	none

The greater part of the oxidation appears to take place during the first year of keeping, as is seen from the following results from examination of first-class copper Mid-Kent hops:—

January 7th . . .	2·61	per cent. tannin.
March 3rd . . .	2·03	,, ,,
October 26th . . .	1·26	,, ,,
December 4th . .	1·20	,, ,,

Moisture.—The normal moisture content of hops is 10 per cent., since hops containing a greater percentage soon come down to this on storage.

Sulphur.—The hop bine during cultivation is often treated with sulphur or solutions containing the same, and during drying in the oast-house the coke fires are sprinkled with sulphur or sulphur is burnt above the fire, in which cases sulphurous acid fumes pass to the hops.

The practice of sulphuring the bines was years ago proposed by Liebig, and there is no doubt that it acts as a valuable germicide. The practice of sulphuring in the oast-house during drying is also good, in so far as the sulphurous fumes here also act as a germicide.

It has not yet been definitely proved, but nevertheless practical experience has shown, that a sulphured hop keeps longer than an unsulphured one; and on the whole, provided sulphur is not employed to excess or to cover defects or disguise the real character of the hops, the practice can hardly be condemned.

Brewers, however, attribute yeast troubles and beer stench to the use of hops containing sulphur, and under these circumstances it becomes necessary to test hops for the same, so that when found the hops may and should be rejected.

Biological Examination.—Behrens estimated the number of organisms and mould fungi in both sulphured and unsulphured hops as follows:—

Description of Hops.	1 Gram of Hops contained	
	Total Organisms.	Mould Fungi.
Unsulphured	13,637,600	422,800
Sulphured	8,056,300	169,200

From this the effect of sulphuring in diminishing the number of organisms and mould fungi is unquestionable, and it follows that sulphur rightly applied is beneficial.

Sugars.

Raw Sugars.

The sugars of commerce may be roughly divided into two classes, Raw and Refined, each class containing many varieties and qualities running imperceptibly one into the other. There is a broad line of distinction, however, to be drawn between the two, for the term "raw sugar" is generally applied to sugar manufactured in a more or less crude manner on the plantation where it is produced, whereas the term "refined sugar" appertains more directly to those finer qualities of sugar which are manufactured

from the raw by subjection to a repeated process of purification, this being generally accomplished at central factories far distant from the country where the sugar is grown, on account of the cost of coal, etc.

In the manufacture of refined sugar it is a matter of some considerable importance to the refiner not only to know how much cane-sugar the sample of raw product which he is going to treat contains, but also the actual amount of cane-sugar which he can obtain therefrom in the process of refining, and which is known as the available amount of crystallisable sugar; for it is now a well-established fact that the presence of salts of mineral and organic acids, as well as invert-sugar and organic bodies other than sugar, exercise a marked influence upon the crystallisation of sugar.

A study of these bodies, and the effects produced by them upon crystallisation, has given rise to a method of valuing raw sugars which has been adopted in France and Germany as well as in this country, and is known as the "rendement," or the net amount of crystallisable sugar obtainable from a given sample of raw sugar. This assumes that for every 1 per cent. of ash contained in the raw sugar, 5 per cent. of cane-sugar is prevented from crystallising; and that for every 1 per cent. of invert-sugar present, an equal proportion of cane-sugar is retained in the molasses. Hence the rule, from the amount of cane-sugar found by polarisation, deduct five times the weight of ash plus the weight of invert-sugar which may be present: the remainder is taken as the refining value or "rendement" of the sample. Thus, a sugar showing an analysis

Cane-sugar	90	per cent.
Invert-sugar	3	,,
Ash	1	,,

would, according to the above method, give $90-(1\times5+3)=82$ per cent. of available crystallisable sugar.

To the invert-sugar manufacturer it does not matter, however, whether there be much invert-sugar present in the raw material or not; he bases, therefore, the value of the sample on the total amount of sugar (cane and invert) which can be obtained. So that a sample which might be altogether rejected by the refiner on account of the high percentage of invert present, would be acceptable to the saccharum manufacturer, provided that the other bodies present, organic and mineral, were not exceptionally high.

Analyses of some of the principal varieties of raw cane-sugar

used in the manufacture of invert-sugar are shown in the following table by Heron :—

	Cane Jaggery.	Cane Jaggery partially refined.	Penang.	Egyptian.
Cane-sugar	75·04	91·07	76·00	80·38
Invert-sugar	11·06	3·04	11·79	4·30
Other organic matter	3·25	0·81	2·60	0·81
Ash	5·46	0·62	2·85	7·32
Water	5·09	4·46	6·76	7·19

Heron says: "As will be noticed, on studying the analysis, all raw sugars contain what, for want of a better name, are termed organic matters other than sugar, or non-sugar; these may be divided into three distinct classes, namely, organic acids, nitrogenous bases, and non-nitrogenous substances. From such substances upwards of sixty definite chemical compounds have been already separated, and their properties determined; most of these are very undesirable bodies to have in malt wort or beer, hence most of the raw sugars should be rejected as unsuitable for the production of even the commonest kinds of ales and stouts."

This is perfectly true, but provided the brewer purchases only the finest of raw sugar, such as the better class, partially refined jaggery or Egyptian, he is perfectly safe in employing it both in common ales and stout, and in inverting it for pale ale production.

When purchasing raw sugars for running mild ales or stout, or for inversion purposes, only those should be selected which will give a solution possessing a rich and luscious flavour. For priming purposes only the very highest class sugars should be employed. The candy sugar frequently used for this purpose, as well as the well-known coffee crystals, are remarkably pure and contain over 99 per cent. of actual cane-sugar.

Beet-sugars should in no instance be employed, as they always communicate objectionable flavours.

Sugar Inversion.

The process of inverting cane-sugar consists in transforming such sugar into dextrose and levulose, two forms of sugar which, when combined, constitute the well-known invert-sugar or saccharum.

The transformation may be brought about by acids, such as sulphuric or hydrochloric, or by certain enzymes derived from animal and vegetable substances—as, for instance, the animal enzymes contained in their intestinal juice, or the vegetable enzymes such as diastase contained in malt, and the invertin or invertase contained in yeast. Whether the transformation is brought about by an enzyme or an acid, the reaction is the same; that is to say, it consists in the assimilation of water by the sugar molecule, followed by its separation into two different kinds of sugar—dextrose and levulose—which, although possessing the same molecular composition or formula, possess very different properties. The formula for cane-sugar, $C_{12}H_{22}O_{11}$ may be written thus:—

$\left.\begin{matrix} C_6H_{10}O_5 \\ C_6H_{10}O_5 \end{matrix}\right\}$ which, when undergoing inversion, splits into two molecules, one of which at the same time takes up a molecule of water (H_2O), thus:

$$\left.\begin{matrix} C_6H_{10}O_5 \\ C_6H_{10}O_5 \end{matrix}\right\} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

Following up this nomenclature, we see that the sugar has already the molecule dextrose contained within itself as it were, whilst the $C_6H_{10}O_5$ molecule becomes hydrated to levulose. Both these sugars are fermentable; but during the stage of fermenting worts, if cane-sugar has been employed, the yeast has first of all to transform it into invert-sugar before it can split it up into alcohol, carbonic acid, etc. It is therefore concluded that if cane-sugar is employed, the yeast during the fermentation of the wort will become seriously weakened by reason of the double function it will be called upon to perform. In 1892, however, this was disputed by J. O'Sullivan, who says; "The power which yeast possesses of producing alcoholic fermentation is not altered in any way by the yeast having first hydrolysed cane-sugar."

The methods of inverting sugar on the brewery premises as carried out by the brewer are by the aid of either acid or yeast (invertase) and are practically as follows:—

Acid Process of Inversion.

A wooden vessel containing wooden rakes is necessary for the purpose, as the acid would act upon the metal if a metal vessel or one containing metal fittings were employed. Water is run into the vessel, the quantity being 0·6 of a barrel for every cwt. of sugar to be inverted. The sugar is then added, rakes started, and

steam injected until a thorough solution is obtained; but the solution is not boiled, as caramelised products might be formed and extract lost. At this stage pure sulphuric acid, sp. gr. 1·84, is cautiously introduced, the quantity being 0·8 of a lb. per cwt. of sugar.[1] The solution is now brought to a temperature of 180° F. (82° C.), maintained at this temperature for 45 minutes or more until such time as a sample, upon being neutralised and tested, shows the inversion to be complete; generally speaking, 45 minutes is sufficient time.

The solution is then neutralised by the addition of milk of lime (calcic carbonate $CaCO_3$, thoroughly mixed with water), the quantity being 1·7 lb. of lime for every lb. of acid used. The solution is now thoroughly roused (rakes going) for about 5 minutes, and is then allowed to stand for about 4 hours, by which time the sulphate of lime, formed by the combination of the acid and lime, will have subsided. The solution is then filtered through a simple bed of granulated animal charcoal and in a perfectly brilliant condition run to the copper, underback, or other vessel.

Great care must be taken in weighing or measuring the acid and in weighing the whiting before adding them to the sugar solution, and it is essential that they should be added very slowly and a little at a time. According to this method, at a temperature of 180° F. (82° C.) from 95 to 98½ per cent. of the sugar should be inverted in 45 minutes. These figures are, however, only theoretical, and such percentage is never obtained in practice. Much of course depends upon the quality of the raw cane-sugar employed. During inversion the acid produces substances termed "inert carbohydrates" to the extent of from 10 to 14 per cent. and often more. These bodies yield weight, and therefore do not lessen the gross extract unless previously filtered out; and being totally unfermentable, they may be looked upon as so much loss.

The extract obtained from brewery-manufactured invert varies with the quality of the raw cane-sugar employed and the ability displayed in manipulation: 72 lbs. per 2 cwt. may be taken as a reasonable average, whilst 76 lbs. may be readily obtained from a really good and well-refined sugar.

[1] To find the equivalent in fluid ounces:—

$$16 \text{ oz. water} = 1 \text{ lb. } \frac{16 \times 0{\cdot}8}{1{\cdot}84} = 6{\cdot}95,$$

therefore approximately 7 fluid ounces of acid may be used per cwt. of sugar, and the trouble of weighing the acid obviated.

Invertase Process of Inversion.

The process of inverting sugar by the aid of yeast (invertase) is indeed simple. Sugar and water, in the proportion of 1 part of the former to 4 parts of the latter, are added to the inversion vessel, rakes are started, and the temperature raised by steam inlets (or preferably steam coil) to from 131° to 140° F. (55° to 60° C.). Such temperature is sufficient to in time dissolve the sugar, and is most favourable to the complete inversion. When the sugar is dissolved, the solution is filtered through a bed of animal charcoal and passed back to the inversion vessel. Fresh, vigorous yeast is then added,[1] the amount being about 1 per cent. on the weight of the sugar, and the solution is kept in agitation, by means of the rakes, for 4 hours. The time specified is sufficient to invert the sugar, but the quantity inverted depends greatly on the total amount of yeast allowed to play upon the solution, and it is therefore impossible to confirm any two sets of experiments conducted with varying measures of surface area, or, in other words, with unlike quantities of yeast. For instance, every brewer is aware that yeast cells, when undisturbed in a liquid, follow the general law of displacing a volume of fluid equal to their own weight; it therefore follows that if two solutions of 5 and 20 per cent. of sugar respectively, were infected with the same quantity of yeast and not kept constantly agitated, the yeast would subside far more rapidly in the 5 per cent. than in the 20 per cent. solution. Taking it, however, that we constantly agitate the fluid, and that the proportions of water, sugar, and yeast as well as the temperature are as stated, complete inversion results in 4 hours.

Such a simple method of manipulation undoubtedly recommends itself, and no prejudice can exist as it does against the use of acid. There are no inert bodies formed, and the whole of the extract, which is that of the original sugar plus the increase due to hydration, consists entirely of invert-sugar. Such extract should be readily obtained in practice to the extent of 88 lbs. per 2 cwt. of sugar.

The speed of inversion increases rapidly with the temperature until 131°–140° F. (55°–60° C.) is reached. At 140° F. (60° C.) the invertase is slowly destroyed, and at 167° F. (75° C.) it is immediately destroyed. At the lower temperature, the speed of action increases with rise of temperature in accordance with Harcourt's law, the rate being about double for 10° rise, but above 30° the increase is not nearly so rapid.

[1] In practice yeast is employed, and not a prepared extract.

Elevated temperatures have no permanent effect on the activity of invertase, so long as they are not sufficiently high to destroy it; and the favourable temperatures to the inversion, viz. 131°–140° F. (55°–60° C.), are beyond the point of the fermentative and vital energy of the yeast organism.

Minute quantities of sulphuric acid (0·20 per cent.) are exceedingly favourable to the action, but a slight increase of acidity beyond the most favourable point is very detrimental. The caustic alkalies, even in small proportions, are also instantly and irretrievably destructive of invertase. As, therefore, all raw sugars vary considerably in these respects, that is to say, some being distinctly acid and others alkaline, the question of manipulating the sugar solution so as to render it slightly acid before adding the yeast, has to be reckoned with, or little or no inversion will take place. This, however, is not a grave drawback provided the brewer prepares himself to meet the requirements with every sample of sugar he manipulates; it being merely necessary to make, for instance, a 10 per cent. solution of the sugar and test it by the acid or alkaline burette.

Invert-sugar.

There are naturally various qualities of invert-sugar on the market, varying with the character of raw sugar originally dealt with, the conditions of inversion, and whether or not final solidification is effected artificially.

A sugar only partly inverted, that is to say, containing an excessive amount of unconverted cane, will not solidify after being removed from the vacuum pans, no matter to what ordinary degree of concentration the sugar has been subjected during the boil.

A large percentage of cane-sugar may not be objectionable, but it points to the fact that the sugar has been incompletely inverted.

The presence of much mineral matter or an excess of unfermentable bodies point to incomplete filtration through animal charcoal, and to the fact that a low-class sugar has been, in the first place, employed.

The better the class of sugar employed for inversion, the more readily will the resulting invert solidify when removed from *vacuo*, hence the solidity is some criterion of the quality of the sugar. It should be remembered, however, that the cheaper sugar—glucose—is sometimes mixed with invert in order to

improve the colour, cheapen the cost of production, and effect solidification. All samples of sugar sold as invert should therefore be tested by the polarimeter for added glucose, and where this is found an estimation of the quantity should be made, and the true value of the sample ascertained.

The colour of the solution should not be excessive, as high colour points to poor purification by filtration through charcoal. The acidity should also be low, as otherwise it points to incomplete neutralisation. The qualities of invert naturally vary with the price, but the well-known No. 1 and No. 2 qualities of different manufacturers should be of a good degree of purity and strength. The following figures show reasonable requirements:—

	No. 1.	No. 2.	
Invert-sugar, not less than . .	74·0	72·0	per cent.
Cane-sugar, not more than . .	1·5	2·0	,,
Mineral matter, not more than .	1·75	2·25	,,
Unfermentable bodies, not more than	3·0	4·5	,,
Optical activity, not less than . .	$-10[\alpha]_D$	$-7[\alpha]_D$	,,

Saccharines prepared from Starch.

Glucose.

Kirchoff, in the year 1811, was the first to prepare glucose or dextrose by the action of sulphuric acid on starch, and although very many modifications have since been introduced, it is still made substantially in this way.

Hydrochloric acid has been found the best for converting starchy materials, but in practice, like with cane-sugar inversion, pure sulphuric acid is used. Other acids are, however, sometimes employed, such as oxalic and phosphoric, and the conversion is often carried on under a pressure varying from two to ten atmospheres. Pressure accelerates the process, but there is the objection that such pressure causes caramelisation, and as a result dark-coloured products are obtained. This is of slight consideration where animal charcoal filtration is afterwards resorted to, because nearly all such inert bodies are thus removed, and decolorisation to a very considerable extent effected; in fact it is only when the solution contains a very large percentage of caramel that the decolorisation is not effectual.

Glucose readily ferments under the action of yeast, requiring no conversion, as is the case with cane-sugar.

It is a comparatively easy matter to accurately value a sample

of commercial glucose. The colour of the solution should not be excessive, as too much colour is a certain sign of either bad material or bad manufacture, or both combined. The solution should be fairly bright, or otherwise it points to the presence of an excess of proteid bodies. The ash should not be much over 1 per cent., as a higher percentage shows faulty filtration. The acidity should be low, or it points to incomplete neutralisation. The extract should not be much under 77 lbs. per 2 cwt. per barrel, that is, it should not contain much more than 10 per cent. of water and no samples should give less than 73 lbs. per 2 cwt. per barrel, that is, should not contain more than 15 per cent. of water.

Samples which contain dextrin always contain more water than when this substance is not present.

The fermentable matter should not be less than 70 per cent., or, if calculated on the dry extract, not less than 80 per cent. of the latter. There is no reason why this should not partly consist of maltose.

It is generally considered that proteid bodies should be absent, or present in small quantities only, for, if even small percentages be present, it is a sure indication that the sugar has been prepared from imperfectly purified material. The percentage of proteid bodies in English maize glucose is about $1\frac{1}{2}$ to 2, and in American glucoses considerably lower, ranging from 0·14 to 0·19 per cent. Continental glucoses, however, are sometimes prepared from potatoes, and in such instances are very objectionable, as they invariably contain traces of the unpleasant alkaloid present in that plant.

Dextrin-maltose, Malto-dextrins or Amylöins.

These substances are prepared from starchy materials on somewhat the same lines as in the manufacture of glucose, the difference being that the acid conversion is stopped at a point before the starch has been hydrolysed to glucose; that is to say, for example, when the product of the reaction has a specific rotatory power of about $[\alpha]_j 193°$, and a cupric oxide reducing power of about 21.

By stopping the reaction sooner, or allowing it to proceed further, or by altering the proportion of acid to water, or the temperature and pressure at which the reaction takes place, the solutions are made to contain various proportions of dextrin and maltose; hence the proportions of dextrin and maltose vary

greatly in these materials. Such products contain very little dextrose or glucose, sometimes none at all, but may occasionally be found to contain unconverted starch, the latter in cases of imperfect manufacture.

The sugar is naturally fluid, and sent out in a clear and nearly colourless condition. Owing to such solutions containing dextrin and maltose to some extent in a combined state as "malto-dextrins" or "amylöins," they are only partially and slowly fermentable. They were much in use ten or fifteen years ago, particularly as priming syrups, on account of the slow and continuous "conditioning" which they are capable of imparting without rendering beers too sweet.

Caramel.

The average colouring power of good caramel is 18° on ·01 per cent. solution examined by the 52 series of yellow glasses in Lovibond's tintometer.

The average density of fluid caramel is 1·377; the average cupric oxide reducing power 33·87 per cent. (as glucose) on the fluid product; and the mineral matter or ash should not exceed 0·80 per cent.

The specific rotatory power and degrees of fermentability should be low.

Most caramels are partially fermentable, but considerable fermentability points to imperfect preparation, since the original sugar should have been converted into unfermentable bodies.

By over-conversion, considerable inert bodies and insoluble carbon are formed, and these are generally greater in solid caramels; they reduce the extract value and tintorial power, but although the carbon has no effect and does not prove the caramel to be a bad one, yet the presence of an excessive quantity of inert bodies is objectionable in view of the fact that little is known of their composition or behaviour when added to wort or beer.

All good caramels when in solution possess a rich flavour which readily distinguishes them from inferior varieties.

Some caramels have liquorice mixed with them, and others various proportions of the extract derived from oats; they are used solely in the manufacture of stout, for which purpose they possess advantages where the particular flavours imparted by them are desired.

PART IX.

MICROSCOPICAL AND BIOLOGICAL.

Microscopical.

The Microscope.—Without doubt a really good microscope is an invaluable instrument to the brewer, and in his hands, provided he thoroughly understands its use, and further, how to diagnose the appearance of the substance he is viewing, it is to him what the stethoscope is to the physician or the compass to the mariner.

There are variously constructed instruments on the market ranging in price according to the actual requirements of the purchaser, but for all necessary brewing and malting purposes a really good instrument may be purchased for about seven or eight pounds.

It is unnecessary here to go into minute details regarding the construction of the various microscopes, and indeed to do so would occupy too great a space; and as particulars can readily be obtained from numerous text-books dealing solely with this subject, we need only briefly deal with the important points.

The compound microscope (Plate VIII.), from a mechanical point of view, consists of a tube A, known as the body tube, upon the lower end of which an internal thread is chased, into which the various objectives B are screwed in turn; or the "nose-piece," as the end of the tube is called, may be constructed with a slide attachment carrying two or three objectives, so that by the mere turning of the attachment the desired objective may be brought into position without the delay of having to take off one objective in order to screw in another. Obviously the sliding attachment is more convenient and saves much time.

Into the body tube A there slides another tube C, termed the draw tube. This is open at its upper end, and into this

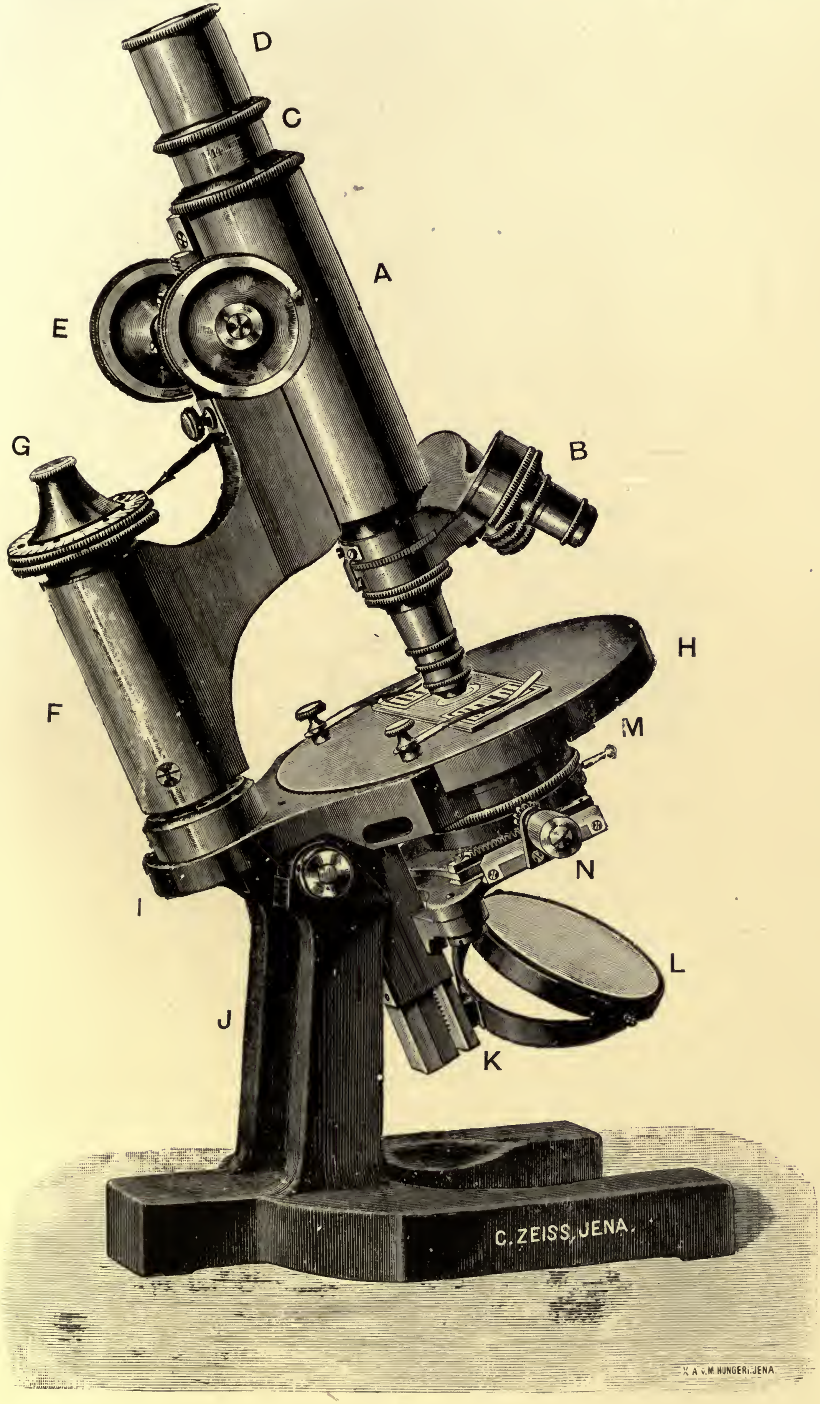


FIG. 80. (½ Full Size.)

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opening the various eye-pieces D are inserted. By means of the draw tube C the length of the instrument can be varied, thus enabling the microscopist to alter the distance between the eye-piece and the objective.

The body tube A is moved up and down by rotating the large milled-head E, and by this movement the "coarse adjustment" of the distance between the objective and object is effected. The pillar F has a triangular base, its movement being effected by an exceedingly fine screw actuated by the milled-head G; this arrangement being known as the "fine adjustment." The triangular pillar is fixed to the stage H, the latter consisting of a brass plate perforated with an aperture immediately under the objective.

The stage H is attached by a movable joint I to the pillar J, which in its turn is fixed to the base of the instrument, the latter being of considerable weight in order to render the instrument stable. Under the stage the tail-piece K is fixed, which on its lower extremity carries the half circle in which the mirror L swings. The semicircular piece is attached to the tail-piece by a screw in such a way that it can be revolved on the axis of the screw; and this motion, combined with the swinging one, enables the mirror to be placed at any required angle. In using the microscope the mirror is placed at an angle so as to illuminate the field of view which is obtained by transmitted light, and obviously there is always a wide variation of the incident beam of light both as regards its angular aperture and its direction.

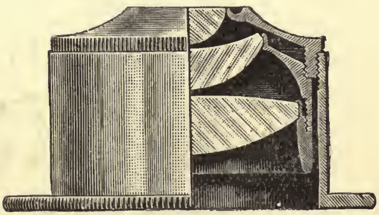

FIG. 81.

To meet this the well-known Abbe illuminating apparatus or condenser M (fig. 81) was introduced in 1872, and is acknowledged as an indispensable accessory, at least in advanced microscopic work.

A further accessory which may be separately employed or combined with the Abbe condenser is the iris diaphragm N (fig. 82, A and B), which facilitates a very gradual variation of the aperture. The smallest opening of the iris has a diameter of about 1 mm., the largest one of 32 mm., so that it may remain in its place even when the condenser is used at full aperture.

The most important portions of the microscope are the quality of the lenses in eye-pieces and objectives, for on their perfection depends the clearness and distinctness of the image viewed.

The most common form of eye-piece consists of two plano-convex lenses having their convex sides directed towards the objective, with a diaphragm between the lenses. It is the simplest and least expensive form of eye-piece, and is suitable for all ordinary purposes; in fact it is the best to use with ordinary objectives, for as these are over-corrected and the eye-pieces under-corrected, the two balance one another and give a correct image.

Other "dry" objectives generally consist of three small achromatic lenses. Each achromatic lense consists of two lenses

A B

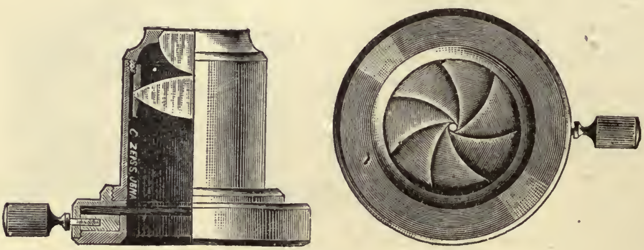

FIG. 82.

of different kinds of glass cemented together, and by this arrangement an image free from colour is obtained.

In addition to ordinary objectives which are designated "dry," another series is manufactured termed "immersion." When using these a drop of water is placed between the cover-glass and the front lens of a "water-immersion" objective, or a drop of cedar-wood oil in the case of an "oil immersion." The object of using an immersion lens is to bring a larger cone of rays into the objective. Light in its passage from the cover-glass to a dry objective has to traverse a layer of air, and rays of light in passing from glass to air suffer refraction, that is, they are bent away from the perpendicular. If, however, a layer of water which has a higher refractive power than air is inserted between the cover-glass and the front lens, then the rays are not bent so far away from the perpendicular, and consequently a larger bundle enters the objective. When a layer of cedar-wood oil, which has the same refractive power as glass, is inserted in

this way, the rays suffer no refraction at all; they continue straight along in their course, and consequently a still larger bundle enters the objective. This increases the angle of the objective and gives better illumination. Owing to the oil employed being of the same refractive power as the cover-glass, an oil immersion is independent, within wide limits, of the thickness of the cover-glass, and for this reason such objectives are called "homogeneous." Many objectives, other than "homogeneous," are provided with what is called a "correction collar," by means of which the upper pair of lenses can be removed nearer or farther from the remaining lens by turning a collar (fig. 83, A and B). This arrange-

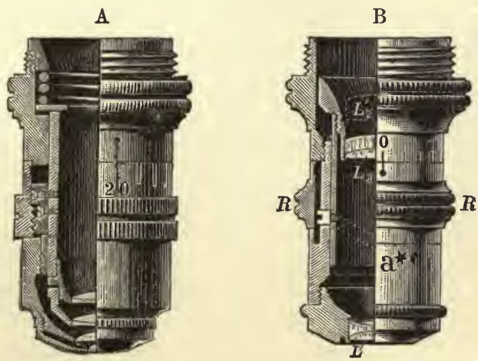

FIG. 83.

ment is for making a correction for the thickness of the cover-glass employed.

Within recent years a new series of lenses have been introduced termed "apochromatic," these being constructed, by means of the most careful mathematical calculations, of special kinds of glass and of a lens of the mineral fluorite; they are, however, exceedingly expensive. They possess one marked advantage, viz., they can be used with eye-pieces of very high magnifying power. Thus $\frac{1}{4}$ inch objective may be used with an eye-piece magnifying 27 diameters, which gives an enlargement of 1080 diameters, as well as with an eye-piece magnifying only 2 diameters, or with any other eye-piece intermediate between these two. This, which gives magnifications of from 80 to 1080 by simply removing the eye-piece, saves trouble in changing objectives.

In differently constructed microscopes the mechanical and

lense arrangements, as will be seen, vary greatly; the Abbe condenser may be of ordinary or apochromatic lenses, and may be separately employed; the iris diaphragm may also be used separately, or both the condenser and iris may be combined. When apochromatic lenses are employed, compensating eye-pieces may be used; and these may or may not also possess an iris diaphragm (fig. 84) and numerous other advantageous mechanism which go to increase the cost of the instrument. Many of these improvements, although necessary in advanced microscopy, are entirely unnecessary for the brewer's work; and, as before stated, a really good instrument, both from the two essential points—mechanical and optical—can be obtained at a price within the range of all.

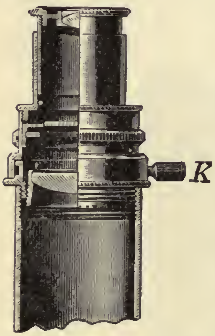

FIG. 84.

Lastly, the various magnifying powers of the eye-pieces are denoted in England by the letters A, B, and C, etc., beginning with the lowest power; the B is, however, most commonly employed, as it is a mistake to use too high a power. On the Continent the eye-pieces are denoted by numbers 1, 2, 3, etc. The length of the tube adopted in Continental instruments is 6 inches, in English 10 inches, and the Continental and English objectives are corrected for these lengths of tubes. Many English microscopes are now, however, made with a 6 inch tube, and a draw tube (graduated) to permit its extension to 10 inches, so they can be used with either make of objective. With a microscope having a 10 inch tube, the total magnifying power of any combination is found by multiplying the magnifying power of the objective by that of the eye-piece. Thus, with an eighth of an inch objective, which magnifies 80 diameters, and an eye-piece magnifying 5 diameters, we obtain a total magnification of $80 \times 5 = 400$. With a 6 inch tube the magnifying power is found by the formula

$$\frac{\text{Objective power} \times \text{eye-piece power} \times \text{tube length}}{10}.$$

With the before-mentioned combination this would equal 240 diameters $\left(\frac{80 \times 5 \times 6}{10}\right)$.

THE SCHIZOMYCETES, HYPHOMYCETES, AND SACCHAROMYCETES, OR BACTERIA, MOULDS, AND YEASTS.

THE SCHIZOMYCETES OR BACTERIA.

All bacteria are extremely minute in size, and although there are hundreds of different species, they nevertheless show but three general forms, which by De Bary have been aptly compared to billiard-balls, lead-pencils, and corkscrews.

Spheres, rods, and spirals represent all forms. The spheres may be large or small, and may be long or short, thick or slender; the spirals may be loosely or tightly coiled, and may have only one or two or may have many coils, and they may be flexible or stiff; but still rods, spheres, and spirals comprise all types.

In size there is some variation, though not very great; all are extremely minute, and never visible to the naked eye. The shapes vary, the diameter of round cells or transverse section of cylindrical ones being generally about 1 μ;[1] the length of cylindrical cells is not commonly more than 2 to 4 times their transverse section, although some cells may attain a diameter as great as 4 μ and occasionally grow to a great length.

The rods may be no more than 0·3 μ in diameter, or may be as wide as 1·5 μ to 2·5 μ, and in length vary all the way from a length scarcely longer than their diameter to extremely long threads. About the same may be said of the spiral forms.

Many bacteria possess the power of locomotion, but this is almost exclusively confined to the bacilli and spirilla, only one motile micrococcus, the *Micrococcus agilis*, being known. The motion is brought about by means of a flagellum, lash or cilium, which they possess at one or both ends or all over their bodies. These flagella keep up a lashing to and fro in the liquid, and the lashing serves to propel the bacteria through the liquid.

During their growth they do not bud as do the yeasts, but multiply by division, and this is the one distinguishing mark which separates the bacteria from the yeasts.

In addition to their power of reproduction by simple division, many species have a second reproductive method by means of spores.

[1] English measurements are frequently given in $\frac{1}{1000}$th of an inch, whereas foreign measurements are in $\frac{1}{1000}$th of a millimetre or micromillimetre (μ), as it has usually been called. As physicists and electricians have used the word micromillimetre to indicate the millionth of a millimetre, the term micron has been suggested to express the thousandth of a millimetre, and the word has been adopted by the Royal Microscopical Society.

The following are those most frequently met with by the brewer:—

Coccus or *Micrococcus* (including chains of *Micrococci*)—
Sarcina.
The viscous ferment.
Bacterium aceti.
,, *Pasteurianum.*
,, *xylinum.*
Micro bacteria (short rods)—
Bacterium lactic.
,, *termo.*
Pasteur's lactic ferment.
,, *butyricum* or *colstridium.*
,, *butyricum* and *B. amylobacter.*
Desmo bacteria (including long and short chains)—
Bacillus subtilis.
,, *ulna.*
,, *leptothrix.*
Spiro-bacteria (spirals)—
Spirillum tenue.
,, *undula.*

Sarcina.—Lindner, who found sarcina in yeast, and using Hansen's moist chamber and gelatine mode of cultivation with decoctions of chopped hay or malt extract, infecting these from various sources, managed to separate and identify several kinds of sarcina of which two varieties are shown (Plate IX., fig. 85, *Pedioccus acidi lactici*, and fig. 86, *Sarcina maxima*).

The former is sometimes found in beer and gives rise to a considerable quantity of lactic acid. The diameter of the single coccus = 0·6 μ to 1 μ. The latter, packet form, is sometimes met with in malt mashes and wort. Diameter, 3 μ to 4 μ.

Sarcina bacteria belong to the slow-growing type; they develop most in wort, in the absence of vigorous yeast, and are found in beer stored for a lengthy period. Low temperatures do not destroy their development. They may multiply to such an extent that the beer becomes ropy, but with rapid consumption and mean temperature they do not cause much trouble.

The Viscous Ferment.—Pasteur speaks of the viscous state of wort and beer, and describes a special ferment (Plate IX., fig. 87) which transforms certain sugars into a kind of gum, together with mannite and carbon-dioxide.

The amount of gum produced does not stand in constant

relationship to the sugar decomposed; it is therefore concluded that there are different viscous ferments, one of which forms only gum. Very little is known of the ferment or ferments, and it is believed by some that the viscosity in liquids, supposed to be formed by one or more special ferments, is, on the other hand, caused by the excreta of bacteria.

Bacterium aceti.—*Mycoderma aceti*, *Bacterium aceti*, or "Mother of Vinegar," as it is variously called, is the organism employed in vinegar factories for impregnating worts intended to be turned into vinegar. The appearance of a film or pellicle on the surface of a liquid is a very ordinary accompaniment of its growth. Plate IX., fig. 88, shows the organism as depicted by Pasteur; it will be seen in the characteristic chain and diplococcus form, the smaller dimension of the latter being about 1 μ. Iodine colours, *B. aceti*, yellow.

Adrian Brown made a very careful investigation of *B. aceti*, taking every precaution to obtain pure cultivations. He described it as forming a greasy pellicle, inclined in the early stages of its growth to climb up the moist surface of the containing vessel. The liquid below the pellicle is usually turbid from suspended cells.

In liquids free from oxygen it does not increase, but keeps alive for a long time. It forms figure 8 cells 2 μ long, united in chains of varying length, and sometimes the chains are composed of distinct cocci. Brown observed abnormal or involution forms 10 μ to 15 μ long, and of a dark gray colour. The shorter rods of cells, when floating freely in a liquid, are motile.

The organism is found in returned and unsound beers and yeast, free exposure to air being the determining factor of its growth. It is also found in bottled ales, sometimes appearing as a film, due to imperfect corking; and often when precautions are not taken to keep the bottles upside down or lying on their sides for some time after filling with beer, so as to allow the beer to absorb the air which would otherwise remain between the cork and the surface of the beer.

A very small quantity of *B. aceti* produces marked acidity in beer, and there is no mistaking the presence of its product, viz., acetic acid, with its highly pungent smell.

Bacterium Pasteurianum.—The above name was given by Hansen to a form of micrococcus which has the same appearance as *B. aceti*; which produces acetic acid, and only differs from it in the sense that it gives a blue coloration with iodine.

Bacterium xylinum.—This ferment was discovered by

Adrian Brown, who describes it as being identical with *B. aceti*. It produces acetic acid, and during growth forms a membrane of cellulose, which sometimes floats on the surface of the liquid. If shaken down, a fresh film is produced from time to time. This appears to be the only form in which the ferment develops, though the membrane may in some cases be dispersed through the liquid, having the appearance of gelatine finings. Viewed microscopically, the ferment shows itself in lines embedded in a transparent, structureless film; the bacterium appears as rods about 2 μ in length, several often being united together.

Sometimes the organism is seen in the micrococcus form, which may possibly be spores; also in long twisted threads 10 μ to 30 μ in length, of a Leptothrix nature. It does not exhibit the large swollen involution forms of *B. aceti*.

Lactic bacteria.—The lactic bacteria, of which there appear to be several species, resemble *B. aceti* in so far as the cells are in the form of the figure 8; but whereas the latter generally form long chains, and only occasionally are distinct cocci found, the former generally occur as distinct small rods contracted as a rule in the middle, forming the figure 8, 2 μ to 3 μ in length.

They are seldom seen in long chains, although they may appear in this form, and it is not unusual to find two or three individuals together forming a small chain. The single rods are motile.

The action of the ferment is the production of lactic acid which, although not imparting any distinct flavour, as is the case with *B. aceti*, produces an intense acidity. Small quantities of lactic acid (1·5 per cent.) retard alcoholic fermentation. Plate IX., figs. 89 and 90, show the ferment first described by Pasteur.

Bacterium termo.—Plate IX., fig. 91, is a typical example of organisms of this class. Whenever a vegetable or animal infusion is left exposed to the air for a short time, this organism, together with others very similar in appearance, invariably make their appearance. They are all motile, having a cilium or flagellum at each end. They have the appearance of small cylinders, each possessing a central constriction giving the figure 8 appearance, and about 1·5 μ to 2 μ long.

Fortunately for the brewer, *B. termo*, like several other organisms to which Hansen gave the generic name of "wort bacteria," is only able to thrive in unhopped wort. The bactericidal action of the soft resins of the hop completely arrests their growth and development.

Bacterium butyricum.—This organism, also known as

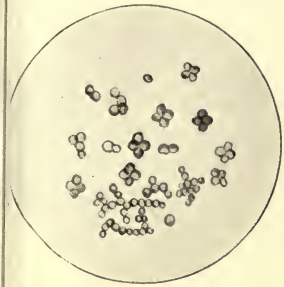

Fig. 85.—Ped. acidi lactici. $\frac{1000}{1}$. (After Lindner.)

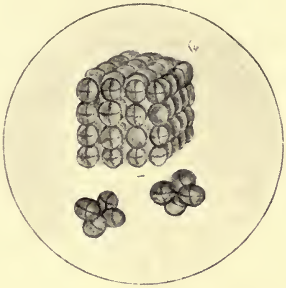

Fig. 86.—Sarcina maxima. $\frac{1100}{1}$. (After Lindner.)

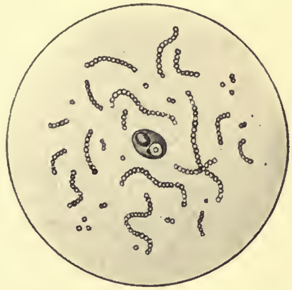

Fig. 87.—Viscous Ferment. $\frac{400}{1}$. (After Pasteur.)

Fig. 88.—Bacterium aceti. $\frac{400}{1}$. (After Pasteur.)

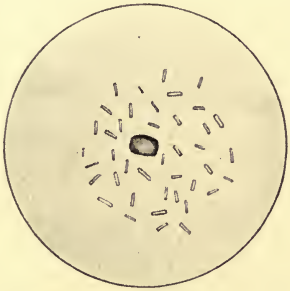

Fig. 89.—Lactic Ferment. $\frac{400}{1}$. (After Pasteur.)

Fig. 90.—Bacterium lactis. $\frac{300}{1}$. (After Pasteur.)

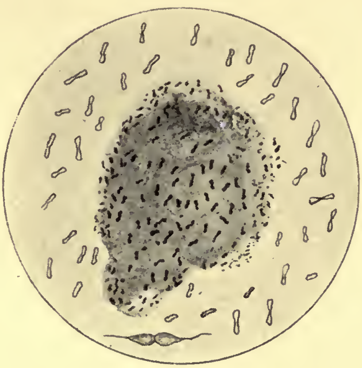

Fig. 91.—Bacterium termo. $\frac{650}{1}$. (After Cohn.)

[*To face p.* 334.

protection. The hay bacillus is therefore a negligible quantity in the brewery.

Bacillus ulna.—This organism, described by Cohn, occurs in long or short, but very broad, cylinders or threads, 2 μ broad, and in a free growth as much as 10 μ long. It is frequently found in infusions such as white of egg. It is occasionally found in yeast and also in beer, but it does not grow in the latter. Plate X., fig. 94, shows the ferment.

Bacterium leptothrix.—Appears as long threads, sometimes of great length, and twisted on themselves. It is found in the slime of pipes and wherever filth is allowed to accumulate, and may often be detected in putrifying sweet wort. It has not been greatly studied, and is possibly only a particular form of *B. subtilis* (Plate X., fig 95).

Spirillum tenue and undula.—These organisms are common in rapidly putrifying liquid and moist substances. *Sp. tenue* has been met with in returned sour ales, and once or twice in forced samples, and both forms in decomposing sweet wort and in water. *Sp. undula* has also been met with in putrid grains, and slime from pipes and the dripping from water-pipes.

Sp. tenue is about 1 μ thick and 4 μ to 15 μ long. *Sp. undula* about 1·4 μ thick and 8 μ to 12 μ long.

Both are looked upon by many investigators as the same species, but the latter has wider spirals and an active movement (Plate X., figs. 96 and 97).

Numerous other bacteria are known, but sufficient has been detailed to give the reader a fair general knowledge of the bacteria, and to show the significance of strict control in regard to brewery cleanliness and germ-laden air-exclusion.

The Hyphomycetes or Moulds.

The hyphomycetes or moulds, commonly called the fungi, are flowerless and leafless plants devoid of chlorophyll (which produces the ordinary green tints of other vegetables), and they fructify by means of cells separated from the tip of certain filaments, or produced within the cavity of the protoplasm. They derive nutriment from the substances on or in which they grow. It is their natural office to promote chemical change in organic structures, and to some extent in inorganic matter as well. They are therefore found accelerating decomposition; according to ignorant belief, springing from it. They are fertilising agents, providing nutriment proper for phænogamous plants. They serve as food for innumerable

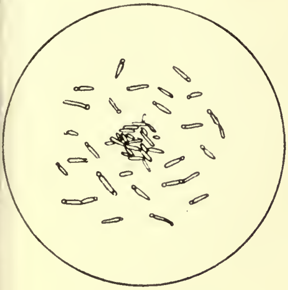

FIG. 92.—Bacterium butyricum. $\frac{350}{1}$.

FIG. 93.—Bacillus subtilis. $\frac{450}{1}$. (After Cohn.)

FIG. 94.—Bacterium ulna. $\frac{400}{1}$.

FIG. 95.—Bacterium leptothrix. $\frac{600}{1}$.

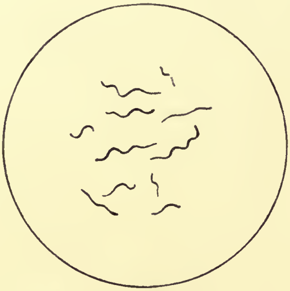

FIG. 96.—Spirillum tenue. $\frac{400}{1}$.

FIG. 97.—Spirillum undula. $\frac{400}{1}$.

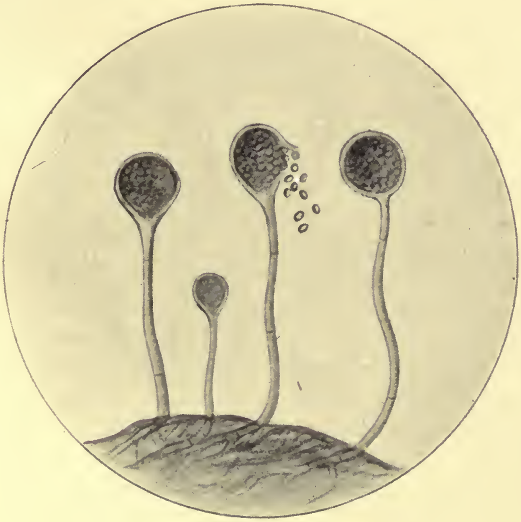

FIG. 99.—Mucor racemosus.

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insects and larvæ. They also check exuberant growth, appearing in many forms as parasites on living vegetable and animal structures. Some of them offer highly nutritious food to man, and others contain essences having medicinal and other properties.

The forms in which fungi appear are very numerous, and are classified into a great many orders and genera; but the lower order are commonly called moulds, and over two thousand British species of them are known.

The mildew which appears on articles of food, and which is familiar to everyone, is seen under the microscope to be an aggregation of elegant and perfect plants, infinitesimal in size, but subject to laws of growth as in higher plants.

Among this vast family of plants, belonging to one class, yet diverse from one another, there is but one kind that the Britisher condescends to regard with favour (*i.e.* the mushroom); all the rest are lumped together in one sweeping condemnation. They are usually looked upon as vegetable vermin only made to be destroyed. With the rapid growth of science during the past two or three decades all this is altered, and whereas in the past but few eyes could see their beauty, their office unknown, their varieties disregarded, and in fact hardly allowed a place among Nature's lawful children, but considered something abnormal, worthless, and inexplicable—they are now being correctly classified, their office perfectly understood, and their industrial use, just as with bacteria, found of considerable benefit in the manufacture of certain articles of commerce.

Fungi are a class of plants governed by modifications of the same laws that control the development of all other vegetables. Each species has a separate existence, and its nature, characteristics, constitution, and inherent properties may vary very greatly from those of even its nearest congener. Fungi do not, any more than the bacteria or yeasts, spring up indiscriminately, spontaneously, or uncertainly. Each species obeys fixed laws of growth and development, and is not transmutable into others. Each species has its own particular locality and habitat, and its characteristics are precisely definable; nor are these liable to greater variation than is the case in flowering plants.

The modes by which fungi are fertilised have yet to be discovered. Some are propagated by means of the mycelium to a certain extent, but the universal method of reproduction is through the medium of spores, which correspond to the seeds of flowering plants. These spores are of infinitesimal size, even in the largest fungi, and are generated in inconceivable multitudes

by each plant. They become productive only when they reach their proper pabulum. The conditions under which they become productive have yet to be learnt. These spores are disseminated in countless myriads by the air, which is ever loaded with them. Some varieties, for example, it is almost impossible to exclude from their special nidus. Other kinds are apparently disseminated by water, by the sap of plants, by the blood and excreta of animals, birds, reptiles, or insects, and in yet other ways.

The mould fungi occupy a low scale in the vegetable world; they are more or less parasitic, and are found on decaying matter in association with bacteria. They secrete powerful enzymes in their tissue, by the aid of which they break down and simplify the complex bodies on which they grow. These bodies in their turn are seized upon by the bacteria, who thus complete the work of destruction. The mould fungi do not entirely confine their attention to the vegetable world, for they often grow and produce disease in animals and insects. For example, the fungus, *Torrubia militaris*, grows on the body of the wasp, and even man sometimes falls a prey to this organism. The spores of the *Aspergillus fumigatus*, taken into the lungs in the act of breathing, adhere to the inner tissue, vegetate, and produce their mycelium, causing the disease *pneumomycosis*; the patient shows all the symptoms of *tuberculosis*, and usually succumbs through inability to absorb oxygen.

The common mould, *Mucor racemosus*, will grow in the inner tube of the human ear, giving rise to *otomycosis*; the membranes of the ear become pierced, and when the nerve centres are reached frightful irritation, madness, and death result.

Examined microscopically, the mould fungi generally resolve themselves into an infinity of delicate filaments filled with living protoplasm often in a state of continuous motion. The filaments are sometimes simple, more often ramified. Grouped together in various ways, they constitute what is known as the "mycelium."

The mycelium liquefies and renders assimilable the matter on which it grows, of which it forms its tissue, and finally produces the spores. The spores or seeds are produced in marvellous numbers in the special capsule or "sporangium." Some of the sporangia contain as many as two million spores. Their formation is generally effected by an asexual process; a certain portion of the mycelium differentiates, becomes more or less spherical, and the spores are produced by internal cell-division. Certain moulds, however, exhibit a real sexual mode of reproduction, such as is met with in the higher forms of vegetable life; two distinct

branches of the mycelium join together and form a conjugated sporangium or large spore (zygospore) capable of giving rise to the mould growth afresh.

The mould fungi, when grown on cereals, attack the cellulose and starchy portions of the grain, transforming them into sugars which are then oxidised, generally with the production of organic acids.

They possess the curious property of being able to adapt themselves to an anaërobic (without air) mode of existence. The genus *Mucor* of which the *Mucor mucedo*, often found growing on the excreta of herbivorous animals, can be taken as a type, all exhibit this capacity.

When the *Mucor mucedo* is placed in a saccharine liquid, a change comes over the mycelium. Transverse septa appear at very close intervals in the filaments; the divisions thus formed become more and more spherical, and finally, detaching themselves from the mycelium, swim about in the liquid. They then reproduce by budding after the manner of yeast; at the same time an alcoholic fermentation is set up in the liquid. The amount of alcohol produced during fermentation set up by moulds does not greatly exceed 3 or 4 per cent., although some species can produce as much as 8 or 9 per cent.

Such yeast forms or "sprouting colonies" are known in an astonishingly wide relationship among the fungi. In fact nearly every one of the large groups is capable of producing yeast forms if submerged in saccharine liquids—*e.g.*, besides many *Mucors*, species of *Mycoderma*, *Chalara*, *Oidium*, *Torula*, and *Monilia* among the doubtful Hyphomycetes; *Exoascus*, *Dothidea*, *Fumago*, *Dematium*, *Bulgaria*, and others among the Ascomycetes; species of *Tremella* and *Exob asidium* among the higher Basidiomycetes, and numerous species of *Ustilago* among the Ustilagineæ, all form yeast colonies under certain conditions. It is owing to this curious behaviour that the controversy has arisen with regard to the nature of the yeast plant. Some naturalists have held that yeast is only the bud or "gemma" form of certain moulds, and it is still believed an open question as to whether the so-called true yeasts, *i.e.* the *Saccharomycetes*, are autonomous forms or merely sprout forms of some higher fungus.

Every brewer is aware of the danger of mould in either the brewery or malt-house, and is familiar with at least the common varieties. In general the moulds are a positive danger, and every care should be exercised in preventing their appearance or growth in any part of the brewery or malt-making premises. Barley,

during its vegetation on the malt-house floor, is often attacked by *Penicillium glaucum*, *Mucor mucedo*, *Aspergillus glaucus*, and other varieties which communicate offensive odours to the grain; and although their growth is arrested or their vitality destroyed at the kiln-drying temperatures, the grain nevertheless contributes nauseous flavours to the beer brewed from it, rendering such beer at times positively undrinkable. The same applies to mouldy hops: the vitality of the mould is undoubtedly destroyed during the boiling of the wort, but the objectionable flavour is nevertheless imparted to the resulting beer. With hops used for "dry hopping" the case is perhaps still more important, since here the mould is not destroyed, and many varieties not only increase and multiply, quickly turning the beer rancid, but the secreted enzymes have a marked effect in reducing the residual beer extract.

During hot weather the mould fungi multiply with remarkable rapidity, and can frequently be detected on exposed sugar solutions, wort, etc., left standing for any length of time. They grow rapidly in beers of low gravity or of low alcoholic strength, when once they gain access, *Monilia candida* being the most prominent under these conditions.

Odium lactis makes its appearance as a thin white skin on the surface of beer, and cask plant left unwashed for any length of time is a fruitful breeding place for several fungi. So also is wooden plant such as fermenting vessels and storage vats, the outsides and particularly the bottoms of which not being easily got at for cleaning. Such vessels are liable to quickly become covered with mould, which, if allowed to remain, ultimately destroys the timber. Damp walls, ceilings, and floors are often seen copiously laden with mould, and the spores are wafted about by atmospheric currents contaminating places which would otherwise not be difficnlt to keep clean. There are then, as stated, an endless variety of moulds, all of which more or less play a significant part and vary in their method of reproduction. It will be sufficient, however, to give brief particulars of a few of the most important common varieties, emphasising the point that, with regard to one and all, the brewer should take every care, by scrupulous cleanliness and antiseptic treatment, to stamp them out whenever they make their appearance, and particularly to see that his pitching yeast and hops for "hopping down" purposes are free from such contamination.

The mould fungi have been divided into five orders:—Hypodermii, Phycomycetes, Ascomycetes, Basidiomycetes, and Myxomycetes.

For a complete account and full details of the various forms of development, reference must be made to botanical and other works;[1] the following, however, are of special interest to the brewer:—

HYPODERMII.

Ustilago carbo (*Mildew*, *Smut*).—Spores, brown, circular; episporium, smooth; sporidia, ovoid cells.

The spores occur as a black powder in the ears and panicles of wheat, barley, and oats.

Tilletia caries.—Spores, round, pale brown; episporium with reticulated thickenings. In germinating, the sporidia grow out radially from the end of the promycelium; these, at their lower part, conjugate by a cross branch and separate from the promycelium, and at some point of the pair a hypha grows out, on which abundant secondary sporidia develop. The latter are long, oval cells, which can in turn germinate. The fungus occurs in the form of a stinking powder in grains of wheat, which renders the meal impure and gives it a disagreeable smell.

Urocystis occulta.—The spores consist of several cells united together: partly, large dark-brown cells in the interior; and outside, several flat, semicircular, colourless cells. The promycelium germinates as in *Tilletia*, but the cylindrical cells produce a hypha, without, as a rule, previous conjugation. They occur as a black powder in rye-straw in long, disintegrated stripes, which are at first greyish. The affected plant produces abortive ears.

PHYCOMYCETES.

Mucor mucedo (Plate XI., fig. 98).—A commonly occurring white mould on rotten fruits, mouldy bread, old yeast, damp barley, malt, hops, etc. It readily makes its appearance upon horse manure and other highly putrefactive organic substances, which it gradually covers with a luxuriant vegetation consisting of long, delicate, silky filaments (fig. 98, *a*, *a*) each of which ends in a small rounded head. The body of the plant consists of a number of branched filaments (*b*, *b*, *b*, *b*) or hyphæ. Some of these ramify into the substratum on which the fungus grows, and the mass so formed is termed a "mycelium."

The mycelia of contiguous plants interlace with each other and form a felted mass. The plant, until the period of spore formation, consists of a single cell formed externally by a thin, much

[1] Sachs, *Text-book of Botany*; Jörgensen, *Micro-organisms of Fermentation*.

branched tube of elastic, transparent, and colourless cellulose. The inside of this tube is more or less completely filled with protoplasm permeated by the cell-juice, and this latter, collecting at various points, forms vacuoles.

The study of the appearance of the spores and their **growth** during vegetation is effected most conveniently by taking up with a piece of platinum wire a mass of sporanges of a growth of the fungus. A sporangia (fig. 98, *c*) is taken up on the wire, and no matter how few spores are collected by the wire, there will be far more than is required for the impregnation of a liquid. A little sterilised and cooled wort is now taken in a watch glass and the wire containing the sporangia used to stir up the wort, by which the sporangia not ruptured spontaneously are ruptured by the stirring. The watch glass is now covered and allowed to stand for one hour, after which the wort is again stirred so as to distribute the spores. From the wort one or more hanging-drop cultures are now made, taking care that each drop does not contain more than one or two spores. The culture is then placed under the microscope and its progress watched from hour to hour.

In course of time it will be noticed that each spore has swelled to about ten times its original size, has become circular, and developed a large vacuole. In the course of a few hours the spore pushes out one or two buds, which gradually increase in length and become hyphæ. The plant, during this development, absorbs more nutriment from the wort than is required to supply the energy necessary for its vital functions, and consequently an accumulation of substances takes place in its interior. Space is provided for this accumulation by the hypha increasing in length, and afterwards by its throwing out side branches. When the lengthening process has proceeded for some time, the membrane at some point on the side of the tube is pushed out by the protoplasm forming a bud which gradually enlarges to form a hyphal branch. This growth and subdivision of the hyphæ goes on until the many-branched mycelium is formed.

In old plants there is a tendency for the hyphæ to become divided into distinct cells by the formation of partition walls, as is the normal course with many other mould fungi.

In the course of **sporulation**, the plant in time puts forth at various points upright hyphæ which are termed "aerial hyphæ" (fig. 98, *a*, *a*), their function being to bear the organs of fructification. Upon each of them attaining a certain length they cease to grow, and the upper ends become swollen into a small cell, the "sporangium," on which minute drops of water appear.

This water is not condensed on the plant, but is actually passed through the membrane by osmotic pressure. A partition now commences to form at the junction of the cell with the stem, the end of which assumes the form of a knob, called the "columella," situated within the cell (fig. 98, *d*).

A portion of protoplasm is in this manner cut off from the remaining portions of the plant, which, apart from its complicated figure, had up to this period consisted of a single cell.

The exterior of the sporangium becomes covered with minute spicules of calcic oxalate, which give it a granulated appearance. The protoplasm in the sporangium now becomes marked out into a number of oval masses, which, though close together, do not actually touch. These eventually become invested with a membrane, and gradually develop into perfect spores (*c*). The rest of the protoplasm, which is not used in forming the spores, is then transformed into a gelatinous substance.

The sporangium, which in its early period of growth is of a pale colour, gradually, as it ripens, turns first to brown, then to black, its outer wall at the same time becoming thin and brittle.

When the sporangium is ripe, the contained gelatinous matter absorbs water, swells up, and the cell wall bursts, resulting in the spores being scattered in all directions.

The interior knob or "columella" (fig. 98, *f*) remains attached to the end of the aerial hypha for some time, often with a portion of the covering of the sporangium attached, which forms a small collar round its base.

The detached spores are now wafted about by air currents until they by chance come in contact with a suitable nutritive medium, in which case they germinate and form new plants of the fungus.

Like all other low members of the vegetable kingdom, the mucors exhibit a method of reproduction common to the higher forms of plant life; that is to say, by **sexual influence**. In this form two cells, the male and the female, are concerned, the process of reproduction being in consequence called "sexual." So long as the mucor vegetates on the surface of a nutritive fluid, it propagates in the asexual manner; but when grown on a solid substratum, as is invariably the case in its natural state, it then reproduces itself in an entirely different manner. When reproduction is about to take place, two short club-shaped branches grow out from contiguous hyphæ which become filled with protoplasm and gradually approach one another until their extremities touch. A cell wall, which cuts off a portion of protoplasm from

that of the branch, now develops in the end of each of these short branches. The cell walls which are in contact now dissolve, and the contents of the two cells fuse together (fig. 98, *g*) and then grow into an enlarged cell called a "zygospore" which, as it ripens, turns black, its exterior becoming covered with excrescences. The zygospore now becomes detached from the rest of the plant, and when placed in a suitable medium commences to germinate after the lapse of six or eight weeks. This lengthy time taken in commencing germination has led to the term "resting-spores," which is also often applied to bacteria and to barley, which is capable of germinating after the lapse of hundreds of years. It is apparently a provision of nature to tide the plant over a period of scarcity of food, for resting-spores are, as a rule, only formed when the supply of food runs short.

On germination the cell wall of the zygospore (fig. 98, *h*) ruptures, and an unbranched hypha grows from it which sends up an upright stem at the extremity of which a sporangium is formed (fig. 98, *j*), the spores of which, when ripe, escape, and reproduce the plant in an asexual manner.

The mucor does not form gemmæ as does *Mucor racemosus*, but its mycelium when submerged in wort is able to excite a feeble alcoholic fermentation yielding, according to Hansen, in about 3 months, 1 per cent. of alcohol by volume, and in 6 months, 3 per cent. This power of causing fermentation is greater in those mucors which form gemmæ.

Mucor racemosus (Plate X., fig. 99, and Plate XI., fig. 100). —Hyphæ upright, short, and branched in some instances; sporangia yellowish to pale brown or grey; columella round or pear-shaped; spores round or nearly so. The external portion of the sporangium is tough and insoluble in water. The fungus, as regards nutrition and reproduction, resembles *Mucedo.* It nevertheless has a third method of reproduction, which has not been observed in all the other members of the same family, viz., by the formation of gemmæ. When this is about to take place, the protoplasm in some of the hyphæ of the mycelium collects at certain points in the tube; transverse walls then form, dividing these portions from the rest of the contents of the tube, converting them into distinct cells. The cell walls then thicken, the intermediate portions of the hyphæ decay, and the gemmæ become detached: each of these, under favourable conditions, develops into a new plant.

When the gemmæ are submerged in a saccharine liquid they set up alcoholic fermentation and throw out buds of a spherical

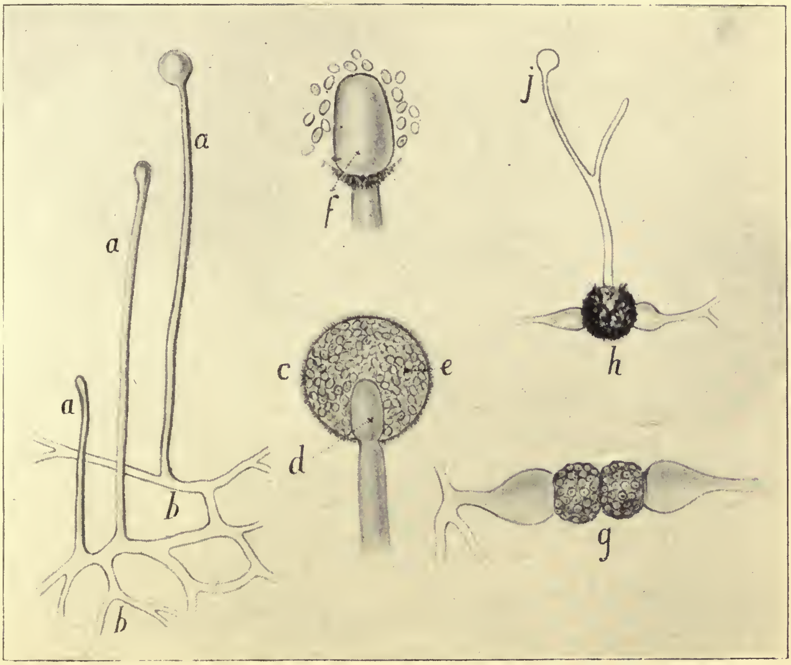

FIG. 98.—Mucor mucedo. (After Brefeld and Kny.)

a, *a*, Aerial hyphæ ; *b*, *b*, branched hyphæ (mycelium) ; *c*, sporangium ; *d*, columella : *e*, spores ; *f*, columella ; *g*, cell-fusion ; *h*, ruptured zygospore ; *j*, formation of sporangium.

FIG. 100.—Mucor racemosus (submerged.)

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or ovoid shape which often hang together in strings or clusters and bear a strong resemblance to yeast (fig. 100); they produce as much as 7 per cent. by volume of alcohol, and secrete an enzyme capable of inverting cane-sugar. It is the only mucor capable of this inverting action.

Other Mucors: Mucor stolonifer (*Lichtheim*).—Mycelium grows in the air and then bends down and re-enters the nutrient substratum; sporangia black, and spores globular.

Mucor circinelloides.—Branching of the aerial hyphæ; the small branchlets bearing the sporangia become considerably curved during development. Like *Mucor racemosus*, it forms gemmæ. It secretes an enzyme capable of fermenting invert-sugar, but is unable to invert or ferment cane-sugar.

Mucor erectus resembles *Mucor racemosus*, but has, like *Mucor spinosus*, spicules on the top of its columella. When grown in the submerged condition it yields, in a wort of a specific gravity of from 1056° to 1860°, as much as 8 per cent. of alcohol by volume: it can ferment dextrin. The fungus also secretes an enzyme capable of hydrolysing starch.

Mucor spinosus has a chocolate-brown sporangia, and the upper portion of its columella is studded with pointed excrescences. When grown under similar conditions as *Mucor erectus*, it yields 5·5 per cent. by volume of alcohol.

Mucor aspergillus (*Lichtheim*).—Fruit-hyphæ thinned at the base, and with many fork-like divisions; dark-brown spores.

Mucor phycomyces (*Lichtheim*).—Mycelium, thick-walled; olive-green fruit-hyphæ; black sporangia, and oblong spores.

Mucor macrocarpus (*Lichtheim*)—Spindle-formed, pointed spores.

Mucor fusiger (*Lichtheim*).—Ovoid spores.

Ascomycetes.

Oidium lactis (Plate XII., fig. 101).—Fruit-hyphæ, simple, erect, and colourless, bearing at their ends a series of chain of conidia. In some cases the fruit-hyphæ branches beneath the chain of spores. Spores are short cylinders. The conidia germinate into filaments of varying length, which by subdivision form septate mycelial hyphæ: these and their branches give rise in turn to spores or conidia. The fungus is deeply stained by the ordinary aniline dyes. In a plate cultivation the colonies appear as white points, and develop into delicate, stellate colonies which ultimately coalesce and form a fine mycelial network covering

the surface of the gelatine. The gelatine is not liquefied. The growth on the surface of agar is similar to that on gelatine. The fungus appears plentifully in sour milk.

Aspergillus glaucus (*Eurotium aspergillus glaucus*).—Mycelium at first whitish, becoming grey-green or yellow-green; spores grey-green, thick-walled. It is found on various substances, chiefly fruit.

Aspergillus niger (*Eurotium aspergillus niger*, De Bary).—Dark chocolate tufts; conidia round, black-brown or grey-brown when ripe. The mould can be cultivated readily on bread moistened with vinegar, on slices of lemon, and on acid fruits and liquids.

Aspergillus nidulans.—Bread and potatoes acquire a reddish-brown colour.

Penicillium glaucum (Plate XII., fig. 102).—Occurs as a white, and later a blue-green mould, on which dew-like drops of liquid sometimes appear. Its spores are present in large numbers in the air, and are liable to contaminate cultivations. The fruit-hypha bears terminally a number of branched, cylindrical cells, from which chains of a greenish conidia are developed.

It is the commonest of all moulds, and most frequently met with during malting operations.

The Saccharomycetes or Yeasts.

Yeast, when microscopically examined, is seen to consist of a number of round or oval bodies, either isolated or joined together in groups, and varying in length from 3 μ to 10 μ (Plate XIII., figs. 103 and 104).

An idea may be gained as to the minuteness of the cells from the fact that a straight row of some 3000 would only equal 1 linear inch, and that the number of cells in 1 ounce avoirdupois of dry-pressed yeast equals about 60,000 millions.

Each separate body is an individual plant which consists of a single cell, surrounded by a thin, transparent cell wall of cellulose or some such closely allied substance.

The interior of each cell is almost completely filled with protoplasm, which in some cells is distinctly clearer than in others. These clear portions are called the vacuoles, and are considered to have their origin mainly in the withdrawal of nutriment from the protoplasm of the parent cell during the reproductive process; the protoplasm being replaced by transparent cell-juice, as Reess terms it, probably of a more aqueous nature than the rest of

FIG. 101.—Odium lactis.
(After Reess.)

FIG. 102.—Penicillium glaucum.
(After Maddox.)

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the protoplasm. At the same time it is by no means certain that the vacuoles cannot appear and be well marked apart from this action. In any case their appearance is very much influenced by varying conditions of temperature and aeration where reproduction does not take place.

Healthy cells usually show at least one vacuole, but often two, and sometimes three. Inside the vacuole may be seen small granules, and these not infrequently move actively in the clear protoplasm.

"Stone square" yeast and specimens of London yeast show this very plainly. The granules are called nuclei. On examining full-sized, well-vacuoled cells carefully, a dark spot called the nucleus may be frequently detected which, on causing the cell to shift its position by touching the cover-glass, pressing it slightly, or in some other way imparting movement, is seen to lie on the cell wall. This is the point at which the bud is appearing when the yeast is in course of reproduction. The nucleus is by no means such a prominent object as it is in most vegetable cells, but it can be rendered visible by staining with osmic acid or hæmatoxylin and picric acid. According to Jansens, the nucleus of the yeast cell divides previous to the division of the protoplasm of the cell taking place, either in the formation of a new bud or of spores.

The cell wall is an integument which, although thin, has considerable elasticity and resisting power. It may, however, be burst by a sudden shock, such as a blow upon the cover-glass, when the protoplasm will be seen to have emerged from the sac. If a little staining agent, such as indigo, is run under the cover-glass, the dead protoplasm of the ruptured cells will take the stain freely, while those of the majority of the unruptured cells remain uncoloured. This rejection of staining matter is peculiar to living protoplasm, for their substance when dead exhibits a powerful attraction for many dyes. The number of dead cells in a sample of yeast may be readily detected by this curious difference in the behaviour of living and dead protoplasm.

Yeast possesses no chlorophyll cells, and cannot therefore obtain the carbon necessary for its nutrition from the carbon-dioxide dissolved in the liquid. This constitutes the main distinction between plants which bear chlorophyll cells and the fungi, to which yeasts belong, which do not. Carbon must be supplied to them in the form of a substance, such as a carbohydrate, which they are able to assimilate. The fungi, being devoid of chlorophyll cells, are dependent upon those plants which

bear chlorophyll cells for their supply of carbonaceous food, and in this respect they resemble animals. In brewing operations the yeast obtains its carbonaceous food supply from the transformed starch which has been originally manufactured by the chlorophyll cells of barley or other starch cereal.

The protoplasmic content of the yeast cell contains nitrogen; this element must also be supplied to the plant in the form which it can assimilate, and yeast manifests certain peculiarities in this respect. The chlorophyll-bearing plants generally are able to derive their supply of nitrogen from a nitrogenous compound of a purely inorganic nature such as potassium nitrate, but the yeast plant is quite unable to do this. If, however, ammonium nitrate is substituted for this salt, then the yeast increases and multiplies, showing that its wants in this direction are satisfied.

The following shows the chemical composition of yeast, which, however, varies greatly in different samples:—

Cellulose	43·3	per cent.
Proteids or albuminoids	44·0	,,
Fat	3·9	,,
Extractive matter	2·5	,,
Ash	6·3	,,

The ash being composed of the following:—

Potassic phosphate	72·6	,,
Magnesic ,,	19·4	,,
Calcic ,,	6·2	,,
Silica, etc.	1·8	,,

Yeast has two distinct methods of reproduction; the first of these, that is, by budding, is by far the more usual one; hence the name "Budding Fungi," which is often applied to the members of this class. The second, and less usual method, is by the production of spores, which form in the interior of the cell. There are numerous species and varieties of yeast.

Formerly the yeasts were classified as species according to the differences in their appearance when microscopically examined. Those of a more or less spherical shape were termed *Saccharomyces cerevisiæ*; those of an elliptical form, *Saccharomyces ellipsoideus*; those of a sausage-shape form, *Saccharomyces Pastorianus*.

No method of distinguishing the species of yeast from its shape alone can, however, be relied upon, as each species is able, under different conditions of cultivation, to assume the forms of others.

The typical examples of brewery yeasts shown from different

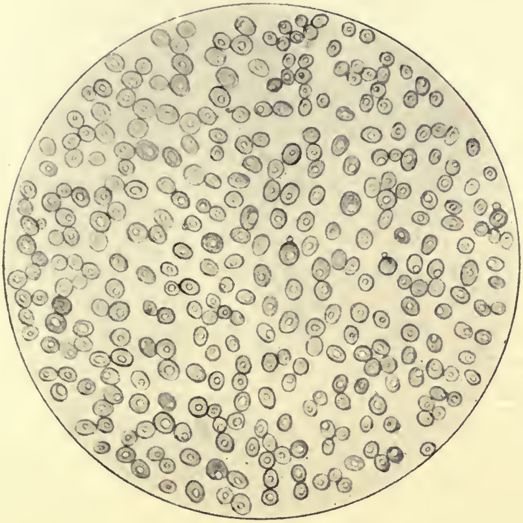

FIG. 103.—Burton Yeast.

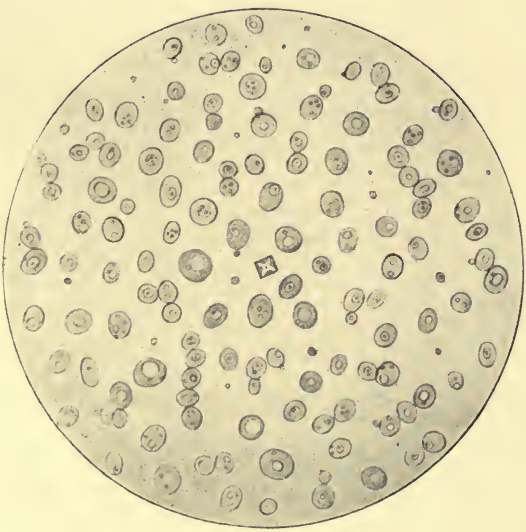

FIG. 104.—London Yeast.

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centres are therefore only cited as examples of what is usually obtained in practice, and that the different methods of fermentation, combined with the character of the saline and other food supplies under normal practical conditions, stamp the physical appearance of a yeast to a great extent.

Although the majority of yeasts are able to ferment the two sugars—glucose and levulose—which require no previous inversion, it is not the case with the disaccharide sugars, maltose, cane sugar, and milk-sugar.

Yeasts such as *S. apiculatus* do not secrete an inverting enzyme, hence they are unable to ferment any of the disaccharides. Other yeasts, such as *S. albicans*, are able to invert and ferment maltose, but not cane-sugar. The *S. kefir* can invert and ferment cane-sugar and milk-sugar, but not maltose. Some yeasts, such as *S. Ludwigie*, are unable to transform any of the sugars into alcohol, but appear to produce oxalic acid instead.

These distinctions are also found in the mould fungi, and their ability to ferment the various disaccharides, or the reverse, depends upon the same causes. *Monilia candida*, for example, secretes invertase.

In addition to invertase, the majority of yeasts, that is to say, the cultivated yeasts, secrete glucase, which, previous to its fermentative action, converts maltose into glucose. Others have the power of degrading a greater or less number of the intermediary dextrins combined with maltose and known as malto-dextrins, which occur in wort, and to this is attributed the difference observable in the attenuative powers of the various yeasts. Again, numerous varieties impart objectionable flavours to beer.

Staining.—Lastly, when examining bacteria, moulds, or yeasts, these minute organisms require some means to be used for rendering their presence more visible under the microscope than when seen in their natural state, and to distinguish them from minute inorganic particles or amongst diseased or healthy tissues; hence some process of staining is generally adopted, and by preference the various aniline dyes are employed.

They easily take the stain of methyl violet, gentian violet, methyl blue, aniline brown, chrysoidin, magenta, rose aniline, etc.; and also osmic acid, iodine in iodide of potassium, and sundry other stains. The blue colours are, however, most valuable and more generally employed as easily differentiating these organisms, whilst iodine is of special value in detecting starch.

Biological.

First we have to consider the means that have been devised for isolating different organisms, since, in order to study the characteristics of any one of them, we must first be able to handle it either alone or at any rate in a fairly isolated condition.

The foundation of the experimental demonstrations of Schwann and of Pasteur lies in the fact that the living protoplasm of nearly all micro-organisms is destroyed—that is to say, undergoes an irrevocable chemical change—when subjected to a temperature slightly below or above that of boiling water. Consequently it is possible, by the action of heat, to destroy the micro-organisms present in an experimental vessel and its contents, and to protect the contents from the further accession of organisms. By this method, and by this method alone, it has been possible to prepare organic infusions, as well as solid gelatine, albumin, etc., which, while capable of supporting the life of organisms, are yet free from their presence for the time being. Such substances are said to be "sterilised," that is to say, we partly fill flasks with wort, meat extract, or other nutritive solution, boil the same, and immediately close the flasks so that atmospheric air may not gain access, and the fluid contents will then keep sound indefinitely.

We may, on the other hand, prepare solid gelatine or albumin in a similar manner, since these substances being liquid when hot, solidify when cold, so that we have only to add them to flasks or tubes, raise them to the boil, and similarly close the flasks or tubes to prevent further access of the atmosphere and its contained germs.

In numerous cases, however, we do not require to keep the flasks or tubes so closed, but on the contrary wish for *pure air* to have access to the contents. In such cases we plug the flasks with sterilised cotton wool or sterilised asbestos, so that the air is filtered and the germs kept back, only pure (germless) air gaining access.

This may also be performed by heating the air or passing it through chemical solutions before allowing it to enter the flasks. We have only then to inoculate these flasks with bacteria, moulds, or yeasts to obtain growths apart from aerial contamination, but a stride further than this is to isolate the organisms so as to inoculate the sterilised nutrient with a special individual organism and obtain its growth independently from any other.

Pasteur was the first to suggest a method by which this could be performed, the idea having occurred to him in his endeavour to test the vitality of yeast.

A few grams of powdered yeast were rubbed up with five times their weight of sterilised plaster of Paris in a sterilised mortar. The mixture was then wrapped up in a piece of sterilised paper and dried at a temperature of 68°–77° F. (20°–25° C.). Two days afterwards a pinch of this powder was sown in sterilised wort contained in a Pasteur flask, and in three days signs of fermentation made their appearance in the wort. When the yeast was two and a half months old the experiment was repeated; this time fermentation commenced on the fourth day, hence the vitality of the yeast was not destroyed, but merely somewhat lowered.

In a similar experiment made with the same yeast when seven months old, the vitality was not altogether lost, but was still more depressed, for it took eight days for signs of fermentation to appear. At the end of ten months the yeast, when again treated in this manner, was found to be completely dead; the wort impregnated with it, though observed for several months afterwards, gave no sign of fermentation.

From this followed a method by which it became quite possible to artificially impregnate the air of a room with yeast organisms. In order to effect this it was only necessary to drop a small quantity of the dried yeast powder from a height, when the presence of living yeast in the air of the room could be demonstrated by opening a series of vacuum flasks in it.

It often happened, in experimenting in this manner, *that one flask received but one organism*; and in this way a *pure culture of a single species of yeast was obtained*, since the whole of it must have sprung from the mother cell.

At the conclusion of a description of these experiments, Pasteur adds the following sentence:—

"Our preliminary observations, although incomplete, seem to favour the idea that numerous varieties of ferments are to be obtained by these means."

Here he distinctly foreshadowed the important field of investigation which has been, in later years, so brilliantly worked out by **Hansen**, and which is hereafter referred to.

The next steps that were taken were more in connection with the bacterial organisms in the atmosphere than yeasts, and unique experiments in this direction were devised by Miquel, Koch, Hueppe, Petrie, Frankland, and others.

Miquel employed, at first, an apparatus which he called an "aeroscope." In this the air to be examined was caused to impinge in a fine jet on a glass plate smeared with a mixture of

gelatine and glucose, which from its sticky nature was eminently adapted to catch and retain the floating particles which the air contained. These particles were then evenly distributed over the glass plate, placed under the microscope, and counted.

Since this method gave no information as to the number of living organisms contained in a measured quantity of air, he devised a peculiarly constructed flask in which a measured quantity of air was caused to bubble through a known quantity of water. He thus obtained the dust of the atmosphere suspended in water, which, after being diluted with a quantity of sterilised wort, was added in small and equally measured proportions to small quantities of sterilised broth contained in a number of flasks. When a large proportion of the contents of the flasks treated in this way exhibited no signs of infection, there was a probability that some of the flasks contained only a single germ; consequently, by taking into account the quantity of air passed through the apparatus, and the number of flasks which became infected, it was possible to form an approximate estimate of the number of organisms that were present in a sample of air capable of development.

Koch devised an entirely different form of apparatus. It consisted of a glass tube about a yard long and two inches bore. One end of the tube was closed with a perforated india-rubber stopper, a glass tube plugged with sterilised cotton wool being inserted in the apparatus in the stopper. A quantity of sterilised gelatine meat-broth was then introduced into the tube and its other end closed with an india-rubber cap. The whole was then sterilised, laid on its side, and allowed to cool. When about to be used the glass tube was connected with an aspirator, the india-rubber cap removed, and air drawn slowly through the tube. In its slow passage through the tube the particles which the air contained were thus deposited on the gelatine. The india-rubber cap was then replaced on the end of the tube and the whole transferred to an incubator, where it was allowed to remain until the organisms had developed and formed colonies. These were then counted and their general appearance noted.

A curious fact was observed in experiments conducted in this manner: the bacterium which had developed was found in that portion of the tube nearest the end where the air entered, whilst the mould fungi were found much farther down the tube; this apparently showing that the specific gravity of the germs of the former organisms is higher than that of the germs of the latter.

Hueppe's method consisted in causing the air under examina-

tion to bubble slowly through warm, sterilised, gelatine meat-broth, which was afterwards spread upon sterilised glass plates. These were placed in an incubator, and the colonies that formed on the same were then studied.

Miquel, Petrie, and Frankland's methods consisted in collecting the floating particles of the air by passing the air through filters containing such substances as sterilised sand, powdered glass, glass wool, asbestos, etc. The material of the filter was then mixed with sterilised gelatine meat-broth and spread on sterilised glass plates; or the sand, etc., with the germs it contained, was placed in a Petrie dish containing gelatine meat-broth, covered over, and placed in the incubator.

The gelatine meat-broth methods of detecting the origin and estimating the quantity of the microbes present in the atmosphere, were shown by Miquel to be far from accurate. He showed that gelatine meat-broth cannot be used at a temperature higher than 75° F. (24° C.), since above this temperature the gelatine becomes fluid, and some bacteria require a higher temperature than this for their development. Many bacteria, again, take a fortnight to form colonies at the usual temperature of the incubator, and by this time so much of the gelatine on the plate is liquefied by the action of certain bacteria, or the plates may be so covered over with the rapidly growing mould fungi, as to be unfitted for further observation. Consequently, those organisms which have not had time to develop entirely escape observation.

Some species of organisms which readily develop in liquids will not do so on a gelatine plate; and finally, there is no absolute certainty that one colony only represents one species of organism.

This was demonstrated by growing colonies on a plate in the ordinary way, then sowing each of them in a separate batch of meat decoction. A second-plate culture was afterwards made with each batch of the infected broth; and, as in these latter colonies species were found which differed from those of the original colony, distinct evidence was afforded that the original colony by no means consisted of one species.

These observations all led to more exact methods being devised which will be understood from the experimental data hereafter detailed under Fractional Dilution.

The result of Pasteur's observations regarding the germs in the atmosphere is that "the air of places in individual proximity to one another may contain at the same time not only the most diverse organisms, but also the most variable quantities of them."

The germs of organisms seem to exist in the atmosphere in the form of clouds, the intervening spaces between which are comparatively germ-free. It is, however, now generally agreed that the number of bacterial organisms present in the air is much modified by the weather; they are most abundant in dry seasons, whilst after a spell of wet weather their number considerably diminishes. The reverse seems to be the case with the mould fungi; these occur in the greater number in the atmosphere in damp weather, and in much smaller quantity in dry weather.

Hansen, who carried out an extensive series of experiments with the object of ascertaining the nature and distribution of organisms in the atmosphere which might exert a prejudicial effect on the operations of the brewer, showed that the yeasts in the atmosphere increase in number from June to August, and are most abundant at the end of August and the beginning of September (the period of the ripening of fruit), after which their number again decreases. Hence it is that at this season the wort, when on the coolers and refrigerators, is likely to receive its greatest contamination from these organisms.

It will thus be realised that the question of aerial contamination is one of vital importance to the brewer, and a vivid picture in this direction is apparent from the experiments conducted by the late Dr G. Harris Morris,[1] in which it was estimated that the number of germs falling upon an open cooler amounted to the enormous number of 142,887 per square yard per hour. The brewer should therefore avoid aerial contamination as far as lies in his power, and the analyst should keep a watch upon any undue contamination, the presence of mould spores or wild yeasts being immediately checked at all costs. A convenient method of procedure on the part of the analyst is therefore given hereafter.

The preceding meat-gelatine method of cultivating micro-organisms having been shown to be unsatisfactory for some purposes, fresh methods had to be devised.

Nageli therefore invented an ingenious method now known as **Fractional Dilution**, which consists as follows:—

Given a liquid swarming with a mixture of various organisms, of which it is estimated by microscopical inspection that one individual in twenty is of the kind it is desired to cultivate; dilute the liquid to such an extent that one drop of it should contain but a single species: it is then probable that every twentieth drop will contain a single isolated individual of the desired organism. Fifty tubes, more or less, of sterilised nutrient

[1] *Jnl. Fed. Inst. Brewing*, 1889, 3, 23.

material are prepared, and into each a single drop of the diluted organism holding fluid is introduced.

One or possibly more of the tubes will thus be inoculated with an isolated example of the desired organism, which will multiply in the sterilised nutrient material and thus yield a pure cultivation which can be recognised by the microscope.

This, practically speaking, led to the initial stage of **Hansen's experiments**, which were briefly yet excellently detailed by Morris[1] as follows:—

"A vigorous fermentation with the yeast from which it was desired to cultivate is promoted in a Pasteur flask (fig. 105); the yeast formed is then largely diluted with a known volume of sterilised water, thoroughly mixed with the water by shaking, and the number of cells in a small drop of the water counted. The counting is effected by means of a hæmatimeter. This may consist either of a microscopic cover-glass on which a number of microscopic squares have been ruled, or of a microscopic glass slide on which the squares are ruled in the centre of a very shallow cell. A good form of the latter is made by C. Zeiss, of Jena, in which the squares measure $\frac{1}{400}$th of a square mm., and the cell is 0·1 mm. deep; the cubical capacity, therefore, of each square, when the cover-glass is on, being 0·00025 mm. The drop is placed well upon the squares, which then assist the eye in counting the cells contained in the drop. Supposing ten cells are found; then, if a similar sized drop is added from the fluid, which has again been thoroughly shaken, to a flask containing a known volume of sterilised water, say, for instance, 20 c.c., the probability now is that this 20 c.c. of water contains ten cells. The flask is thoroughly shaken for some time, and then 1 c.c. of the liquid quickly introduced into each of twenty flasks containing nutritive solution: there is now in all likelihood one cell in each of ten of these twenty flasks. This is of course only a probability. In order to be sure that some of the flasks contain only one cell, it is necessary to allow them to remain until a growth appears. Directly after adding the 1 c.c. of the diluted yeast to the flasks, they are thoroughly shaken and placed in the incubator at the required temperature, the cell or cells present then sink to the

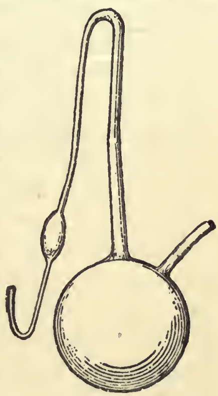

FIG. 105.

[1] *Jnl. Soc. Chem. Ind.*, 1887, 113, 123.

bottom of the flask, remain and grow where they settle, which, in the latter case—*i.e.* if more than one cell is present—is probably at different points on the bottom. After some days the flasks are carefully examined and the points of growth noted. In those flasks in which there is only one speck of growing yeast visible, the inference is that it proceeds from one cell, and consequently the flask contains a pure cultivation; in other cases it is possible that two or more specks are visible; then it is safe to conclude that more than one cell was sown in the flask, and its contents are consequently rejected.

This method has yielded good results in Hansen's hands, especially with ferments which have some distinguishing characters, such as *Sacch. apiculatus.* This method also gives much better results than the one about to be described when it is wished to separate weak and strong yeasts which are growing together in a nutritive liquid. Another point in its favour is the complete absence of any possibility of outside contamination after the 1 c.c. of diluted yeast has been added to the flask.

Hansen, however, afterwards adopted a solid medium for such cultivations, viz., hopped wort and gelatine; and he gives the preference to this material, except under the circumstances mentioned above, since it enables the experimenter to directly observe the individual cells under the microscope, and to follow the course of their development. He adopted a modification of Koch's gelatine-plate method, taking, however, more elaborate precautions to prevent contamination after the inoculated gelatine is spread on the plate. In order to prepare a pure cultivation of yeast, we take a growth of young and vigorous cells, dilute this down very largely with sterilised distilled water in a small Chamberland flask until the proper dilution is reached (this is ascertained by a microscopic examination), and then again dilute a drop of this with sterile, beer-wort gelatine (hopped wort of about 1058 sp. gr. with 5–10 per cent. gelatine), until we have an extreme dilution. A drop of this is then withdrawn with a sterilised glass rod, and spread upon the under side of a thin cover-glass, which is then quickly placed on the ring of a Böttcher's moist chamber. Fig. 106 represents one of the chambers in question: *a* is the thin cover-glass, with a layer of gelatine on its under surface *b*, and placed on the glass circle *c*, which is 30 mm. in diameter and cemented to the glass slide; *d* is a thin layer of sterile distilled water. The inoculated gelatine having been spread on the glass circle and allowed to set, the chamber is then placed on the stage of the microscope and examined.

One or two well-isolated yeast cells are picked out, and the position of these marked on the glass circle by a marker of some description. The whole is then placed in an incubator at about 68° F. (20° C.) and allowed to remain for a day or two. At the end of about two days the growths are generally visible to the naked eye, and appear as small, whitish specks about the size of pins' heads. These specks should be well separated from each other on the glass circle. When the specks have attained a sufficient size they are transferred to sterile hopped wort, of about 1·058 sp. gr., contained in Pasteur flasks. These consist of flasks with the neck drawn out and bent over, as shown in the figure, and with short side tubes, which are closed with a piece of india-rubber tubing and a glass rod; the bent tube is closed with a plug of sterilised asbestos. The transference is effected by quickly lifting the cover-glass, with the colonies, plunging a short piece of sterilised platinum wire into the colony, and then

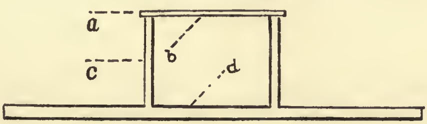

Fig. 106.

immediately dropping this into the side tube of the flask, the glass-rod stopper being quickly withdrawn and replaced. Having got the colony *which we know is derived from one single cell* into the flask, we are then in a position to study its characters and properties.

Such is the process which Hansen has employed to effect a revolution in the study of the Saccharomycetes. By its means he has succeeded in separating a number of apparently different species of yeasts, from which he has selected six for further study.

In addition to these he has isolated two varieties of ordinary bottom-fermentation yeast, which are at present used in the brewery of Old Carlsberg, and also in a very great many other breweries on the Continent. He has also determined that the form, the limits of size, and the appearance of the cells do not remain constant for each variety of species, but are influenced by different conditions of growth. The form and phases of development of the cells, however, when viewed from another standpoint, give very important differences for each variety. This is the case when the cells of the different varieties are exposed to similar conditions, as in the ascospore and film formations: it is found

then that the different yeasts behave in a very different manner, and each species gives well-defined characteristics. This can only be explained by the supposition that the different varieties or species have distinct, innate properties.

We will now proceed to consider the differences which Hansen has found between the different varieties of yeast. He has, as stated, differentiated six species of yeast, which he calls :—

Saccharomyces cerevisiæ, I.
Saccharomyces Pastorianus, I., II., and III.
Saccharomyces ellipsoideus, I. and II.

The ordinary sedimentary forms of these are shown in Plates XIV., XV., and XVI., figs. 107 to 112.

It will be seen that, although the varieties taken separately appear quite distinct, yet if they were mixed it would be extremely difficult to detect one from the other; for instance, the *S. Past.* when mixed with *S. cerev.*, or the *S. ellip.* when mixed with *S. Past.* or *S. cerev.* The size of the cells varies considerably with the species.

The characteristic which Hansen chiefly relies upon in differentiating these species is the ascospore formation. The formation of ascospores in yeast cells has long been noticed, Reess, Engel, and several other observers having described it, and attributed this formation to various causes. Reess built up a system of the Saccharomycetes based upon the form and size of the cells and spores. Brefeld concluded that only "wild" or "natural" yeast was capable of giving spores, whilst cultivated yeast had lost this property. Hansen [1] examined his six species for this formation as follows :—A small quantity of the yeast was spread on a sterilised gypsum block; this block was then placed in a flat-covered glass dish and was kept moist by filling the latter half full of water. The formation was then generally seen when the dish had stood for a few days at the ordinary temperature. The spores generally form as round bodies within the cell, and are usually accompanied by the "sheath-wall" formation. Plates XVII., XVIII., and XIX., figs. 113 to 118, show the formation for the six species. Hansen investigated the influence of different temperature upon the rate of formation of the spores, in order to determine whether the different species could be distinguished from each other in this way. For this purpose it was necessary to know :—

(1) The limits of temperature, *i.e.* the highest and lowest temperatures at which spores were formed.

[1] *Meddelelser fra Carlsberg Laboratoriet*, 1883.

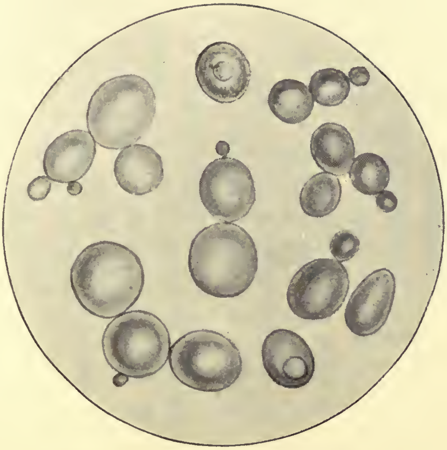

FIG. 107.—Saccharomyces cerevisiæ, I.

FIG. 108.—Saccharomyces Pastorianus, I.

[*To face p.* 358.

FIG. 109.—Saccharomyces Pastorianus, II.

FIG. 110.—Saccharomyces Pastorianus, III.

[*To face p.* 358.

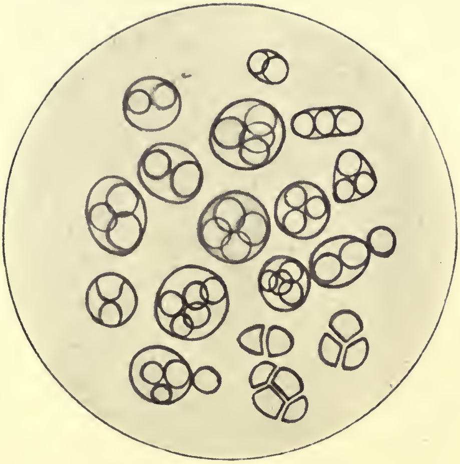

FIG. 113.—Spore Formation.
Saccharomyces cerevisiæ, I.

FIG. 114.—Spore Formation.
Saccharomyces Pastorianus, I.

[*To face p.* 358.

FIG. 115.—Spore Formation.
Saccharomyces Pastorianus, II.

FIG. 116.—Spore Formation.
Saccharomyces Pastorianus, III.

[*To face p.* 358.

(2) The most favourable temperature at which the spores were formed.

(3) The ratio of the intermediate temperatures.

The results obtained (which are given in Table I.) showed that the formation of spores proceeded very slowly at ordinary temperatures, but more rapidly as the temperature rose, until it reached a certain point. When this point was passed, then the formation

TABLE I.—ASCOSPORE FORMATION.

Temperature.		*S. cerev.* I.	*S. Past.* I.	*S. Past.* II.	*S. Past.* III.	*S. ellip.* I.	*S. ellip.* II.
°F.	°C.						
99·5	37·5	None	...	...	...	...	...
96·8–98·6	36–37	29 hours	...	...	...	...	...
95	35	25 ,,	...	...	...	...	None
92·3	33·5	23 ,,	...	...	...	None	31 hours
88·7	31·5	...	None	...	...	36 hours	23 ,,
86	30	20 hours	30 hours	...	...	...	...
84·2	29	...	27 ,,	None	None	23 hours	22 hours
81·5	27·5	...	24 ,,	34 hours	35 hours	...	...
79·7	26·5	...	...	...	30 ,,	...	...
77	25	23 hours	...	25 hours	28 ,,	21 hours	27 hours
73·4	23	27 ,,	26 hours	27 ,,	...	...	...
71·6	22	...	...	...	29 hours	...	...
64·4	18	50 hours	25 hours	36 hours	44 ,,	33 hours	42 hours
61·7	16·5	65 ,,	...	...	53 ,,	...	...
59	15	...	50 hours	48 hours	...	45 hours	...
51·9–53·6	11–12	10 days	...	77 ,,	...	...	5·5 days
50	10	...	89 hours	...	7 days	4·5 days	...
47·3	8·5	None	5 days	...	9 ,,	...	9 days
44·6	7	...	7 ,,	7 days	...	11 days	...
37·4–39·2	3–4	...	14 ,,	17 ,,	None	None	None
32·9	0·5	...	None	None	...	...	...

again decreased, until it at last ceased entirely. The lowest temperature found for the six species was 23°–26·6° F. (½°–3° C.); the highest 99·7° F. (37·5° C.). The highest and lowest temperatures for the different species were also different, and also the limits of temperature within which the ascospore formation takes place in the different species. We see from the table that the differences at the high temperatures, and down to 77° F. (25° C.), are almost inappreciable; but when we lower the temperature the differences become more marked. For instance, at about 51·8° F. (11° C.), *S. cerev.* first shows ascospores at the end of ten days, whilst *S. Past.* II. shows them at the end of seventy-seven hours, and so on with the other species. In making

this comparison, it is necessary to make the experiments with each of the six species under exactly the same conditions, since the use of old or young cells, composition of the nutritive medium, etc., exercise a marked influence on the temperature and rate of formation of the ascospores. Upon these results Holm and Poulsen[1] have based a method for the practical analysis of brewing yeast. Hansen found that the ordinary bottom-fermentation yeast only formed spores at 77° F. (25° C.) after some days, whilst, as we have seen, the "wild" forms, as exemplified by the six species we are considering, form ascospores at this temperature in a few hours. Working with pure cultivations of each species, Holm and Poulsen found that they were able to detect 0·5 per cent. of *S. Past.* I. and III., or *S. ellip.* II., in a mixed yeast; and, as Hansen has shown, that when these "wild" yeast forms, which are the cause of the diseases in bottom-fermentation beer, are present in a barm to the extent of not more than 2·5 per cent. of the total yeast, they do not develop their particular form of disease; it will be seen that, for bottom yeast at least, the ascospore formation forms a valuable means of determining the purity of a barm.

The next and most recent of Hansen's observations are those of the "film" formation.[2] The formation of films on the surface of the culture liquid is peculiar to most micro-organisms when the greater portion of the food material contained in the liquid is consumed. As the result of a series of exhaustive experiments with the six foregoing species, Hansen has established differences in their film formation, both as regards the limits of temperature within which it is possible for a growth to take place, and also in the appearance of the cells of the film; whilst, as a general rule, all the cells of old films show a remarkable change of form, large mycelium-like cells in ramified colonies being formed, yet the cells of *S. cerev.* I., *S. Past.* II., and *S. ellip.* II., in a young state, show no mycelium-like colonies. *S. Past.* I. and III., and *S. ellip.* I., however, show them very early. Table II. shows the temperatures and the length of time necessary for the formation of the films of each of the six species. At the high temperatures there is very little difference in the forms, excepting in the case of *S. cerev.* I. and *S. ellip.* II.; but the young films at lower temperatures, 55·4°–59° F. (13°–15° C.), show very marked differences, and allow the various species to be easily distinguished.

S. Past. II. and *S. Past.* III., which are both top-fermentation

[1] *Meddelelser fra Carlsberg Laboratoriet*, 1886.

[2] *Ibid.*

forms, and the cells of which are very similar under ordinary conditions, show a marked difference at this temperature. Plates XX., XXI., and XXII., figs. 119 to 124, show the various forms of the cells of the films of the six species at 55·4°–59° F. (13°–15° C.).

These are the principal scientific results obtained by Hansen in his researches on the morphology and physiology of pure-cultivation yeasts. It is an open question how far these different yeasts can be considered to represent distinct species, since it is at present a moot point where the bounds can be drawn between

TABLE II.—FILM FORMATION.

Temperature.		*S. cerev.* I.	*S. Past.* I.	*S. Past.* II.	*S. Past.* III.	*S. ellip.* I.	*S. ellip.* II.
°F.	°C.						
104	40	..	..	..	..	..	None
96·8–100·4	36–38	None	..	..	..	None	8–12 days
91·4–93·2	33–34	9–18 days	None	None	None	8–12 days	3–4 ,,
78·8–82·4	26–28	7–11 ,,	7–10 days	7–10 days	7–10 days	9–16 ,,	4–5 ,,
68–71·6	20–22	7–10 ,,	8–15 ,,	8–15 ,,	9–12 ,,	10–17 ,,	4–6 ,,
55·4–59	13–15	15–30 ,,	15–30 ,,	10–25 ,,	10–20 ,,	15–30 ,,	8–10 ,,
42·8–44·6	6–7	2–3 months	1–2 months	1–2 months	1–2 months	2–3 months	1–2 months
37·4–41	3–5	None.	5–6 ,,	5–6 ,,	5–6 ,,	None	5–6 ,,
35·6–37·4	2–3	..	None	None	None	..	None

species and varieties in the Saccharomycetes. On this account Hansen has preferred to give his yeasts the above distinguishing numerals instead of renaming them, leaving this latter until more is known on the subject.

Jörgensen, in his book on *Die Microorganismen der Gärungsindustrie*, attempts to classify the Saccharomyces and sums up Hansen's six species as follows :—

Saccharomyces cerevisæ, I. — A top-fermentation yeast, giving excellent results in practice; used in the breweries of London and Edinburgh in an impure state; develops ascospores at temperatures between 51·8°–98·6° F. (11°–37° C.); film formation at 55·4°–59° F. (13°–15° C.); the predominant number of the cells resemble the original yeast.

Saccharomyces Pastorianus, I.—Gives a bitter flavour to beer; develops ascospores at temperatures between 37·4°–86·9° F. (3° and 30·5° C.); film formation at 55·4°–59° F. (13°–15° C.); fairly numerous, strongly developed, mycelium-like colonies of very elongated, sausage-shaped cells.

Saccharomyces Pastorianus, II. — Causes no disease in beer; develops ascospores at temperatures between 37·4°–22·4° F.

(3° and 28° C.); film formation at 55·4°–59° F. (13°–15° C.); oval and round cells predominant.

Saccharomyces Pastorianus, III.—Cause of yeast-turbidity in beer; develops ascospores at temperatures between 47·3°–82·4° F. (8·5° and 28° C.); film formation at 55·4°–59° F. (13°–15° C.); strongly developed colonies of sausage- or thread-shaped, mycelium-like cells.

Saccharomyces ellipsoideus, I.—Yeast of grapes; develops ascospores at temperatures between 45·5°–88·7° F. (7·5° and 31·5° C.); film formation at 55·4°–59° F. (13°–15° C.); greatly ramified and strongly developed colonies of short and long cells; ramifications often forked.

Saccharomyces ellipsoideus, II.—Cause of yeast-turbidity in beer; develops ascospores at temperatures between 46·4°–93·2° F. (8° and 34° C.); film formation at 55·4°–59° F. (13°–15° C.); resembles the ordinary form in a marked degree.

In addition to these, various other yeast forms have been described by various observers, viz., *S. exiguus* (Reess), *S. minor* (Engel), *S. conglomeratus* (Reess), and so on, but they have not yet been put to the test of pure cultivation. There is another yeast form, already alluded to, viz., *Saccharomyces apiculatus*, which possesses great interest on account of its being the only alcoholic ferment whose cycle in nature has been exactly determined, and since it formed, as already stated, the starting-point in Hansen's researches on the yeasts. It lends itself especially to this purpose, since it has a peculiar shape which is possessed by no other yeast. It forms typical, citron-shaped cells which do not yield endogenous spores, and therefore, correctly speaking, it does not belong to the genus Saccharomyces. The ferment is found upon all ripe, succulent fruit, in the yeast of wine, and also in the spontaneously fermented Belgian beer. *S. apiculatus* is a bottom ferment which is capable of setting up alcoholic fermentation in beer. The fermentation is, however, slight, only 1 per cent. of alcohol being formed instead of 6 per cent. formed by *S. cerevisæ* under similar conditions. The explanation of this is, that it does not ferment maltose, and does not secrete any invertase. In dextrose solutions it sets up a vigorous alcoholic fermentation. Microscopical examinations of ripe, succulent fruit in summer show that this ferment is present in considerable quantity in a healthy, budding condition; on the unripe fruit, leaves, etc., it is not found, and no trace of the ferment can be found upon the plants in the winter. Hansen has, however, shown that the ferment hibernates in the earth

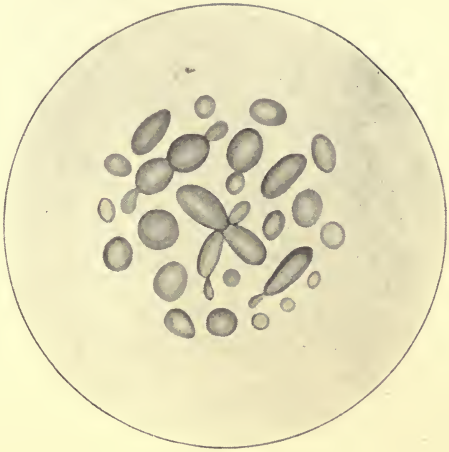

FIG. 119.—Film Formation.
Saccharomyces cerevisiæ, I.

FIG. 120.—Film Formation.
Saccharomyces Pastorianus, I.

[*To face p.* 362.

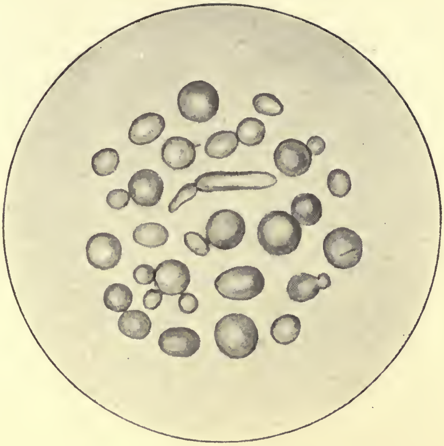

FIG. 121.—Film Formation.
Saccharomyces Pastorianus, II.

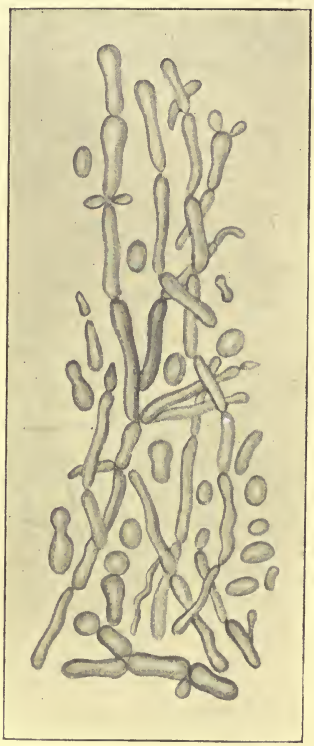

FIG. 122.—Film Formation.
Saccharomyces Pastorianus, III.

[*To face p.* 362.

under the trees, and in early summer is carried again into the air, and on to the ripe fruit by the action of the wind and insects.

Now, what have been the practical results of this work of Hansen? Mention has already been made of the fact that pure-cultivation yeast is in use at the Carlsberg breweries. In 1883 Hansen, having had occasion to study the causes of some cases of yeast-turbidity, came to the conclusion that the only real remedy for diseases of beer caused by "wild" yeast was to work in all cases with yeast which could be guaranteed free from these wild forms. This can only be done by Hansen's method of pure cultivation, or some modification of this method.

Hansen[1] succeeded in isolating from the beer which was submitted to him, by the method described, three varieties of yeast — *S. cerevisæ* (ordinary bottom yeast, which constituted the greater portion), *S. Past.* III. (a form of bottom yeast), and the *S. ellip.* II. (a form of top yeast).

Experiments carried out with the pure yeasts showed that fermentations with the *S. cerev.* gave a beer which was quite free from any form of disease, but that when either one or both of the other forms were also used in the proper proportions, the disease was set up. Further experiments showed that the yeast-turbidity was not caused if the two "wild" yeasts were not added until the end of the primary fermentation. Also, that the disease did not show itself if *S. Past.* III. or *S. ellip.* II. formed 2·5 per cent. only of the yeast used for pitching, and the fermentation carried on in the fermenting cellar until the beer showed an attenuation of 6·7° Balling, and the resulting beer stored for at least three months. If, however, the attenuation was not run down so low as this, and the storage not continued so long, the disease showed itself with the above proportion of "wild" yeast.

The result of these experiments was that Hansen cultivated two varieties of bottom *S. cerev.* for use in the Old Carlsberg brewery, which are known as Nos. 1 and 2. These yeasts, which under the microscope appear to the uninitiated to be identical, give very different results in practice.

No. 1 gives a beer well adapted for bottling, and containing less CO_2 than No. 2. The beer should remain bright in bottle for at least three weeks; it has also a lower attenuation than No. 2. This yeast is chiefly employed for home use.

No. 2 gives a good draught beer, containing more CO_2 than No. 1; it is not adapted for bottling, and is much preferred by

[1] *Zeitschrift f. das gesammte Brauwesen*, 1883, 477.

German brewers to No. 1, and is therefore chiefly cultivated for export.

Now, a word as to the cultivation of pure yeast upon a sufficient scale to barm brewery vessels. It has been shown that it is comparatively easy, with experience and a rigid adherence to the small precautionary details, to obtain a small quantity of pure yeast, but the question arises—How are we to carry on the cultivation under conditions of purity until we have sufficient pure yeast for our fermenting square? Well, it is done by transferring the yeast from a small flask to a number of larger flasks, and when the growth of yeast is over, dividing the yeast in these between a still greater number of larger flasks; taking care, of course, to use all due precautions, as many as fifty or more 1½ litre Pasteur flasks being used for the last laboratory cultivation. This, however, only gives about 2 lbs. of fairly thick yeast. It is then necessary to work with still larger vessels: this is done in the brewery, and as, fortunately for the Danish brewers, they are not hampered by any excise regulations in the breweries, they are able to arrange small fermenting rounds in such a way that they can collect in them sterilised wort, and after adding the yeast, lock the vessels up and adopt means to prevent aerial contamination.

The employment of these pure yeasts is coming very largely into use in the beer-drinking countries of the Continent, and some of the most noted brewing technologists have given it their support, notably, Jacobsen, Aubry, Marz, Lintner, etc. The latter sums up the question in the following statements:—

"1. By contamination with so-called 'wild' yeasts, an otherwise normal brewery yeast can be rendered incapable of producing a beer of good flavour and with good keeping properties.

"2. A contamination with 'wild' yeasts may be produced by the dust of the air during summer and autumn, by the malt, or other sources.

"3. By employing Hansen's method of pure cultivation and analysis, it is possible to obtain from a contaminated yeast a good brewery yeast in a state of purity.

"4. Yeast cultivated in a state of purity possesses in a marked degree the properties of the original yeast before contamination, as far as concerns the degree of attenuation, the flavour, and keeping properties of the beer.

"5. There exist different varieties of normal bottom yeasts (*S. cerev*), each with special properties, which, like the peculiarities of species, are maintained constant.

"Sufficient has already been done to prove that in ordinary brewery yeast we also possess a mixture from which, by Hansen's method, several varieties of *S. cerev.* can be separated, which cannot microscopically be distinguished from each other, but which, when used upon a practical scale, give entirely different results, both as to flavour, brightening, attenuation, and mode of separation of the yeast.

"Experiments have also shown that these characteristics can be maintained unimpaired throughout a very great many successive fermentations in the brewery."

Having thus far paved the way by showing the rapid strides that have been made, we have now to give a few details as to the general apparatus employed and the precautions taken during manipulation, and follow with details of the usual biological methods.

It is hardly necessary, from what has gone before, to state that in all biological examinations it is absolutely necessary that all vessels and apparatus—flasks, test-tubes, beakers, filter papers, cotton wool, glass rods, etc., to be used—must be thoroughly sterilised by heating to at least 248°–302° F. (120°–150° C.), that is, at a temperature which is sufficient to kill all micro-organisms and their spores, which may have settled from the air upon and within the vessels, etc. In the case of large vessels such as flasks, this is conveniently done by passing the flame of a Bunsen burner over the surface of the vessel until it is hot, and then immediately closing the mouth and side tube with sterilised cotton wool and india-rubber tubing and glass rod.

For small vessels, test-tubes, beakers, cultivation chambers, etc., an air-bath, heated to the required temperature, is most convenient. This should be maintained at 248°–302° F. (120°–150° C.), and the vessels allowed to remain in it for two to three hours at that temperature. The cotton wool for plugging the mouths of flasks and test-tubes must also be well sterilised by being loosely pulled out and then heated in the air-bath to the above temperature for some hours on several successive days. Filter-papers used for covering the cotton-wool plugs should also be heated in the air-bath.

Forceps, pipettes, and all other odds and ends of apparatus must be sterilised by being passed through a Bunsen flame at the moment of use.

The solutions most commonly employed for cultivations are rendered sterile and ready for inoculation in the test-tubes and small flasks in the following way:—The solid material, as broth-

gelatine, beer-wort gelatine, etc., must be poured into the previously sterilised flasks and test-tubes, whilst still in a fluid state, and then sterilised by being boiled twice or three times on successive days. Tyndall has pointed out that the spores of certain bacteria are not killed by boiling, therefore it is necessary to boil on successive days in order to give the unkilled spores an opportunity of developing between each successive boiling, when, of course, the next boiling kills the developed organism.

No test-tube or flask containing nutritive material can be considered sterile until it has been kept at a temperature of 89·6°–100·4° F. (32°–38° C.) for at least seven days without any sign of growth appearing. An excellent form of steam steriliser is Petrie's, which enables flasks, tubes, etc., to be sterilised in a current of steam at a pressure of one atmosphere. It consists of a cylinder of steel plate coated with lead, with door fitting air-tight, and covered with felt to prevent radiation. The shelves are of tinned iron and the supporting tables of wood. It possesses a series of Bunsen burners and a steam generator.

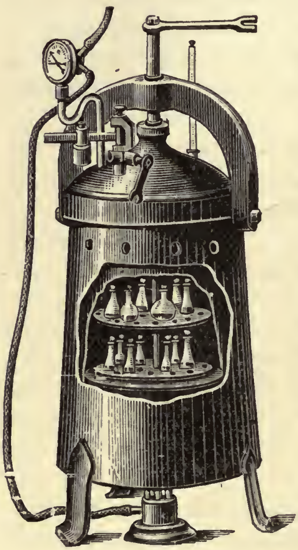

FIG. 125.

Fig. 125 shows an autoclave or high pressure steam digester which may be employed for sterilising as well as numerous other purposes. It possesses a copper boiler, bridge clamp, and central screw, cover of phosphor bronze, safety-valve, steam stop-cock, and manometer. The outer cover is of sheet iron.

Sterilisation is effected by the agency of steam under pressure, in which condition it possesses a higher temperature than when existing at the ordinary atmospheric pressure. Water is placed in the bottom of the vessel to a depth of a few inches, the shelf-holder with its articles introduced, the lid screwed down, and heat applied from below by means of a large Bunsen burner.

Since water always boils at a certain temperature for a particular pressure, the heat of the interior is ascertained from the indications of the pressure gauge. The autoclave is a very useful form of apparatus when samples of beer are to be sterilised. If

such samples are heated in the ordinary steam steriliser, a loss of alcohol takes place. By conducting the operation in an autoclave, and using beer of the same alcoholic strength as the samples, freed from carbon-dioxide in order to prevent frothing, instead of water for charging the apparatus, such samples may be sterilised without loss of alcohol.

For the majority of purposes, however, tubes, flasks, etc., may be conveniently sterilised by washing them first with concentrated

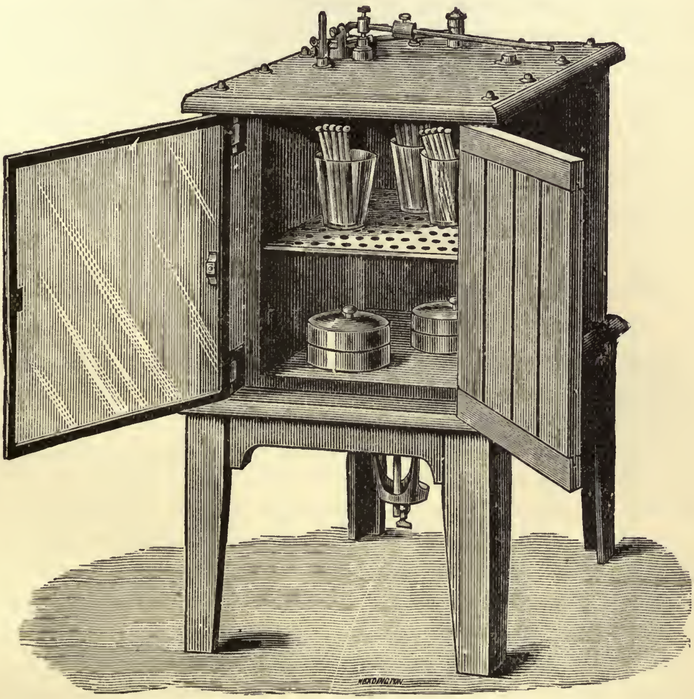

FIG. 126.

sulphuric acid, afterwards with distilled water, plugging them with cotton wool, and placing in the ordinary air-bath, heating intermittently to 248°–302° F. (120°–150° C) for from two to three hours.

Apart from the ordinary forcing tray—which is well known as large copper tray about 4 inches in depth, containing water, placed in a cupboard free from draughts and the water maintained at a constant temperature of 75°–80° F. (23·8°–26·6° C.) by means of a thermostat—the more compact Hearson's incubating apparatus (fig. 126) may be conveniently employed.

This consists of a vessel of sheet metal such as copper, having double walls, and provided with a glass door protected by an outer wooden one; the space between the walls is filled with water. The outer case and stand are of pine, and a patent gas regulator is employed so that the interior of the apparatus may be kept at the required temperature.

It will be as well to point out the necessity of having some place set apart entirely for pure-cultivation work, as the success or failure of these operations depends greatly upon the freedom of the air from germs at the time the experiments are being carried out. For this reason the room should be allowed to remain perfectly undisturbed for some hours before the cultivation is made, in order to allow the atmospheric dust to settle.

The foregoing precautions being observed, we may proceed with typical examples of biological work usually carried out by the brewer's analyst.

Biological Methods. Aerial Contamination. Survey of Brewery and Malting Premises.

The analyst from time to time throughout each year should make a biological survey of the brewery premises in order to detect any undue infection.

To do this, a number of flasks are prepared containing sterilised wort or meat gelatine. The flasks, usually of wide mouth, are thoroughly cleansed, labelled, and the superficial area of the mouth ascertained and noted on the label. They are then sterilised and the nutritive solution added, after which they are plugged with cotton wool and again sterilised. Upon removal from the sterilising apparatus, each flask is held under a stream of cold water and turned and twisted about until the gelatine is spread over the surface in a thin film and has become solidified. Caps of sterilised paper are now tied over the plugs, and the flasks are taken to the yeast store-room, fermenting-room, cooler, refrigerator, malt-house, or other locality where the observation is to be made. The caps and plugs are here removed, the plugs being placed temporarily inside the caps, and the flasks left standing in the open for one hour. The plugs and caps are then replaced, the particulars concerning each flask written on the label, and the flasks taken back to the laboratory so that the germs which have fallen into them may be cultivated by incubation. The latter is performed by placing the flasks in the incubator and maintaining them for two or three days at a

constant temperature of 68° F. (20° C.), by which time the organisms will have fed upon the nutritive material contained in the flasks and have formed colonies in the form of white or coloured specks on various parts of the solidified gelatine. These are now counted; and by taking into account the area of the mouth of each flask and the number of colonies formed, a simple calculation gives the number of germs falling per square inch or per square foot, etc., from the air in the locality where the flasks were opened. On the other hand, the individuals comprising the colonies can be taken up by a piece of platinum wire and either cultivated and their action on wort, beer, etc., noted, or they may be directly examined microscopically.

Biological Examination of Water.

Koch's Method.—For this purpose Koch's "plate culture" method is conveniently employed. Plates of ordinary glass are used, of such a size as can be easily examined under the microscope, their breadth being determined by the distance between the centre of the objective and the pillar which supports the body of the microscope, and they may be about twice as long as they are wide. A convenient size, however, is 3 × 5 inches. The plates are first thoroughly sterilised. An

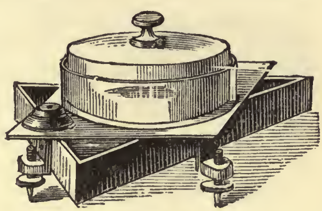

Fig. 127.

apparatus devised by Koch is now used for spreading the mixture and water and nutritive gelatine on the glass plates. This apparatus is shown (fig. 127). It consists of a wooden tripod-stand, the feet of which are formed of three screws by means of which the apparatus can be accurately levelled. The shallow glass tray, shown on the top of the glass plate, is filled with water and ice and placed on the tripod-stand. This is covered with the glass plate on which the culture plate is to rest; and on this the bell glass for covering the cultivation plate and protecting it.

In using the apparatus, the glass plate which is to receive 10 c.c. of meat gelatine is taken out of the steriliser and reversed on to the glass plate on the top of the inner dish under the bell-shaped cover. After melting the gelatine, which should be done without going to a higher temperature than 95° F. (35° C.), the plug of the test-tube is removed and 1 c.c. of the water, after

thoroughly shaking, introduced by means of a sterilised pipette, avoiding as far as possible a vertical position of the tube.

The bell-shaped cover on the levelling apparatus is now raised just enough to allow the mixture to be spread on the glass plate, where it sets in about a minute. As the area has to be measured if the colonies are too numerous to be counted all over, the gelatine should be spread in the form of a rectangle.

As soon as the gelatine has set on the plate, it is at once removed in its dish from the levelling table and placed in the incubator and maintained at a constant temperature of 68° F. (20° C.). The period of incubation generally varies from three to five days, but sometimes it is continued for a longer period of time to make sure that all the organisms present have had a due opportunity of developing.

The gelatine plates are daily inspected during the period of incubation, without removing the glass-cover, so that the progress of the colonies derived from the individual organisms may be watched. They will differ considerably in appearance: some will appear as white, slimy-looking spots resting on the surface of the gelatine, others will have spread more considerably amongst the surrounding gelatine; some may have an iridescent appearance, others may be brilliantly coloured.

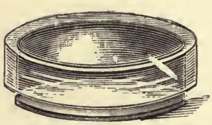

Fig. 128.

Many of the colonies will be found to have left the gelatine film intact, others to have liquefied it, and various degrees of liquefaction may be observed amongst the different colonies. When, however, they have reached fair dimensions, and before the contours of different colonies have begun to coalesce, the plates should be withdrawn for examination.

The Moist Chamber.—Instead of adopting Koch's apparatus as previously described, that known as the moist chamber method may be employed.

This consists of two glass dishes as shown (fig. 128). The lower dish has a diameter of about 8 inches and a depth of about 4 inches. The outer dish is slightly wider, and is used as the cover. The dishes are first thoroughly cleansed and then sterilised in a metal box as shown (fig. 129). They are then smeared internally with glycerine, which, as first shown by Tyndall, retains any bacteria which falls upon them. A layer of filter paper, moistened with thirty or forty drops of a saturated solution of mercuric chloride (a powerful germicide), is placed in the bottom of the narrower dish.

The water contained in this solution keeps the air in the apparatus saturated with moisture. A stand which will hold several cultivation plates, and which may also be used for the before mentioned Koch's method, may be here employed, so that several cultivations may be simultaneously carried on. It consists

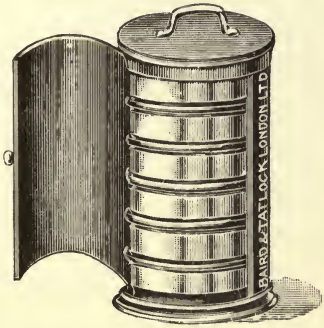

FIG. 129.

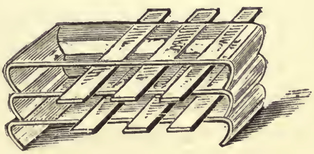

FIG. 130.

of glass plates as shown (fig. 130). When a number of these are superimposed, they form shelves on which the cultivation plates can be placed. The moist chamber containing the cultivation plates is then placed in the incubator.

Counting the Colonies.—For this purpose the apparatus invented by Wolfhügel (fig. 131) is generally employed, which consists of a wooden base, surmounted by a blackened ground

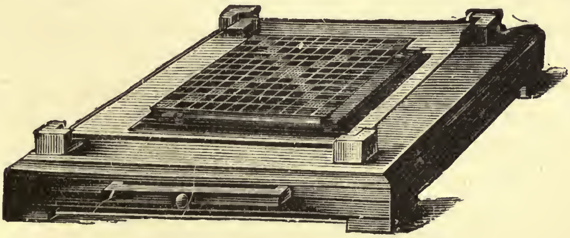

FIG. 131.

glass plate, on which the cultivation plate, withdrawn from the incubator, is placed, and over this is a glass plate marked into squares with a diamond. This plate is supported at the corners in a manner which allows the cultivation plate to be introduced or withdrawn without the gelatine film coming in contact with it.

The sides of the squares on the counting plate are a centimetre ($\frac{3}{8}$ inch) long, and several of these squares are further subdivided.

The counting is performed with the assistance of a magnifying

glass; the number of colonies in a certain number of squares being counted, and the average contained in each square thus estimated. The number so found, multiplied by the number of squares which are equal to the size of the gelatine film, gives the number of germs contained in the quantity of water used for infecting the gelatine.

Petri Dish Method.—This is a much more simple method than the plate-cultivation methods previously described. It consists in taking two Petri dishes similar to those shown (fig. 128), but considerably smaller, one of which fits over the other.

The dishes are usually about 4 inches in diameter and half an inch in height. They are thoroughly cleansed and sterilised, and then the warm, infected wort or meat gelatine is run into the lower dish and spread over the bottom in a thin layer; the upper dish, which forms the cover, being immediately placed on after adding the nutrient, and the whole then left until the gelatine solidifies. The dishes are then removed to the incubator, and when the colonies are formed they can be examined under the microscope and preparations made from them just as when a cultivation plate is employed.

In order to count the colonies, the dish is placed on a sheet of black paper ruled in squares with Chinese white.

Hansen's Method.—Hansen has shown that many organisms found in water which are capable of developing on a gelatine-wort plate will not grow in wort or beer, and that the vast amount of organisms found in water have no significance whatever for the brewer, as very few are capable of vegetating in either wort or beer.

His method consists in taking fifty Pasteur flasks, each having a capacity of about 20 c.c. These are divided into Groups I. and II. Into each of the twenty-five flasks of Group I., 10 c.c. of wort are introduced; and into the remaining twenty-five which constitute Group II, the same quantity of beer. All the flasks are then plugged with cotton wool and sterilised.

When the contents have cooled, one measured drop (0·004 c.c.) of the water under examination is added to each of fifteen of the flasks belonging to Group I., and also to fifteen of the flasks of Group II.

To each of the remaining ten flasks of each group 0·25 c.c. of the water is added. All the flasks are then agitated, so as to distribute the organisms, and are then incubated for fourteen days. At the end of this time the flasks are examined for turbidity, and those which exhibit this sign of infection counted.

Taking the total quantity of water added to the ten flasks, the number of flasks infected, and the total number of flasks taken, we estimate the number of organisms presumably present in this quantity of water, and from this is calculated the number present in each cubic centimetre of the water. Thus if five out of ten wort flasks (Group I.), to which 0·25 c.c. of the water had been added, have become turbid, then, as the total amount of water added to these ten flasks was $0·25 \times 10 = 2·5$ c.c., this quantity presumably contains five organisms capable of development in wort; consequently, the water contains two such organisms per cubic centimetre. If, as sometimes happens, the whole of these ten flasks have become turbid, no conclusion can be formed from them. We then turn to the remaining fifteen flasks of each group, to each of which only 0·04 c.c. of water had been added, and apply the same method of calculation. The same process of calculation is applied to the flasks in Group II., and the number of organisms per cubic centimetre of water capable of developing in beer similarly estimated.

Biological Examination of Malt.

This test was formerly much in vogue, but of late years has been more or less discarded; still it is extremely useful, and gives a good idea of the stability of a wort and the character of the beer that may be expected from it. It is a test, however, that is only reliable in the hands of one who has had considerable experience of the manipulation of malts containing varying percentages of caramelised grain, and kilned off at varying temperatures.

In order to perform the test, a test-tube of about 300 c.c. capacity is taken, a hole is knocked out of the bottom, and the tube is then rotated over a Bunsen flame so as to permanently round off the hole according to requirements. The hole of this tube is now plugged with a piece of cotton wool and the tube then placed inside a similar but slightly larger tube. A circle of cotton wool is now plugged at the mouth, between the inner and outer tube, 250 c.c. of distilled water are added to the inner tube, and this is then plugged at the mouth with cotton wool. The tubes are now placed in the sterilising apparatus and sterilised, after which they are removed, and the contents cooled down under a stream of tap-water. The tubes are then placed in the water bath and allowed to remain until the 250 c.c. of water have risen to the temperature at which the water in the bath is

maintained, viz., 150° F. (65·5° C.). Of the malt to be examined, 25 grams are now weighed and ground, the plug of cotton wool is removed from the mouth of the tube and the ground malt added, after which the cotton-wool plug is immediately replaced. The tubes are again placed in the water bath and allowed to remain, with occasional shaking, for 1 hour; they are then removed and cooled.

We have now a miniature mash, and by slowly raising the inner tube (keeping the circle of cotton wool in its place) the wort filters through the plug of cotton wool in the hole at the bottom of the inner tube and is collected in the outer tube.

When the whole of the wort has filtered through, the inner tube is quickly withdrawn, and the outer one then plugged with cotton wool.

By these means we practically get a 10 per cent. wort in one tube and the grains from the same in the other, the manipulation having been accomplished under strictly biological conditions.

The tubes are now removed to the incubator and inspected at the end of 12, 24, 36, 48, and 60 hours.

According to the number of malt samples it is intended to test, so a number of tubes, prepared as described, are required. Each tube is labelled and particulars noted thereon.

The following is a typical result of such examination :—

BIOLOGICAL TEST.

Condition of Miniature Mash after			
24 hours.	36 hours.	48 hours.	60 hours.
Sound.	Sound.	Cloudy.	Putrid.

Worts taken from the mash-tun taps may be examined by incubating in sterilised test-tubes, and their keeping qualities similarly noted.

BIOLOGICAL EXAMINATION OF HOPS.

This is generally performed by taking 2 grams of an average sample of the hops, together with 100 c.c. of distilled water, in a tube prepared as in the biological examination of malt just described, incubating the same, and noting the colour, flavour, and biological aspect of the liquor at the end of three days.

A plate-cultivation of the infusion may be made, as already described, under water, and the incubated colonies counted and microscopically examined. Naturally, any mould there may be in hops is destroyed during the boiling of the copper wort; but in purchasing hops the presence of mould is decidedly objectionable, since, apart from its tendency to communicate nauseous flavours to the wort, such hops have been badly grown, packed, or stored.

A further method is to employ wort in the tubes instead of water. In such instances it is advisable, for purposes of comparison, to always reduce the wort to a gravity of 1050°. One hundred c.c. of the wort are added to the tube and sterilised, the contents then cooled, and 2 grams of the hops added, the plug of cotton wool being removed for the purpose and immediately afterwards replaced. The tube is then placed in the incubator, and at the end of four days the smell is carefully noted, the attenuation of the wort determined, and after shaking and straining off the hops, the sediment is microscopically examined.

Biological Examination of Yeast.

From what has gone before it is obvious that, from a microscopical examination of yeast, we are enabled to detect the presence or absence of bacteria and the several species of the same; and obviously the presence of more than two bacterial germs in a field of 100 cells, that is to say, more than 2 per cent., is objectionable, showing that greater care should be exercised in the cleansing of the brewery plant and the exclusion of contaminated air. In like manner we are enabled to detect the presence of mould, the appearance of which should be sufficient to at once condemn the sample for pitching purposes.

We are also enabled, by microscopic examination, to detect whether the outline of the cells is thin and the yeast in consequence young and vigorous, or whether it is thick and therefore old, or whether there are granulated cells showing signs of exhaustion by reason of being speckled. The general appearance of the cells, clearness or transparency, boldness, method of clinging together, number in bud, aspect of vacuole, etc., are all well-known signs in estimating the condition of the sample.

Again, we are enabled to detect the presence or absence of any undue proportion of amorphous matter, which, if present, points in many cases to imperfect hop-back filtration of the wort or the

retention of too much of the amorphous matter, which is thrown up during the early stages of fermentation, and in most cases removed and run to waste rather than that it should become intermingled with the outcrops to be used for pitching. We are also enabled, by microscopic examination, in conjunction with the use of staining agents such as indigo, to detect the presence and number of dead cells, more than 2 per cent. being decidedly bad, and also by the use of iodine to detect the presence of starch, the latter being important where flour and salt "dressings" are employed.

These are the only points, however, which the microscope can tell. We are not enabled by its use to detect with certainty the presence or absence of "wild" yeasts, and, as we know from the brilliant researches of Hansen that many yeasts communicate objectionable flavours to beer, it becomes important, particularly where brewing results are not brilliant or are in any way out of the normal, that a complete examination of the pitching yeast be made, and this naturally necessitates a biological examination. We have already seen from Hansen's experiments that spore formation and film formation are necessary in this respect, and therefore it becomes essential to isolate the cells of our pitching yeast, cultivate the same in sterilised wort, and also compel them to live under conditions which form spores and films. From the indications thus obtained we are then in a position to state with certainty the different species which compose our stock, and whether our troubles are attributable or not to the presence of one or more species of wild yeasts.

Biological Examination of Beer.

The application of this examination, generally known as the "forcing tray" test, devised by Horace Brown, is of extreme value to brewers, particularly where an export trade is done, since by its adoption information is obtained as to the stability of beers and whether they will remain bright and in condition under adverse circumstances such as they are expected to be subjected to during their voyage to foreign climes. We are thus able to discriminate as to which beer out of a number of brewings is best able to withstand the necessary adverse conditions; and in like manner with home trade, we are able to judge which beer will stand the longest storage, is best for bottling, and which, on the contrary, it is best to send out without delay.

The beers about to be examined should be carefully drawn from

casks,[1] through freshly bored peg-holes, into capacious Pasteur flasks which have been previously sterilised and plugged. From these flasks samples are then drawn into Pasteur flasks of small size, also previously sterilised and plugged, and placed on the forcing tray and maintained at a temperature of 75°–85° F. (23·8°–29·4° C.).

At the time of placing samples on the forcing tray the specific gravity of the remaining portions of the samples, after filling the small Pasteur flasks, are tested, and also the acidity. A note is made as to the condition of the samples, namely, their brightness, flavour, etc. The samples are left on the forcing tray at the forcing temperature for a period, as follows :—

Stock pale ales—3 weeks.
Running beers—10 days.

At the end of these respective periods the beers are removed from the tray and the following observations made and noted :—

Flavour.
Degree of brightness.
Specific gravity.
Acidity.
Amount of deposit.
Microscopical examination of deposit.

The following is a copy of note-book, with examples, which will be found useful in recording results :—

[1] It is not unusual to take samples from the racking tanks at the time of filling the casks, but it should be remembered that the results of the forcing of such beers are not as reliable as with samples drawn direct from shipment casks, since in the latter case the beers will have been dry-hopped, the resins acting to a large extent as a preservative, and that the interior of trade casks, although no doubt scrupulously clean, are not sterile. On the whole, more reliable results are therefore obtained by dealing with the beers direct from the casks in which it is intended to send them out. It is also sometimes advisable to force beers after the addition of salicylic acid, so as to gain an idea of their keeping qualities under the influence of this antiseptic.

FORCING-TRAY RESULTS.

Number of Sample.	Date Sample was placed on Tray.	Quality of Beer.	Condition and Flavour.	Specific Gravity.	Acidity.	Date off Tray.	Amount of Deposit.	Microscopical Examination.				Remarks.
								Yeast.	Secondary Yeast.	Bacteria.	Mould.	
26	6/4/06	XXXX.	Bright.	1013°	0·10 per cent.	16/4/06	Small.	Chiefly primary. No dead cells.	Very few Sac. Past.	2 per cent. B. lactic.	None.	Sound.
28	8/9/06	EIPA.	Brilliant.	1014°	0·98 per cent.	29/9/06	Very large.	Primary. Chiefly secondary. 4 per cent. dead cells.	Large number Sac. Past.	5 per cent. B. lactic. 3 per cent. Sarcina.	None.	Very unsound.
30	9/10/06	AK.	Cloudy.	1012°	0·12 per cent.	30/10/06	Very little.	Primary. 2 per cent. dead cells.	None.	Few B. lactic.	None.	Good.
45	7/12/06	IBA.	Brilliant.	1010°	0·14 per cent.	28/12/06	Little.	Large amount, chiefly primary. 3 per cent. dead cells.	Few.	Few B. lactic.	None.	Good.

PART X.

APPENDICES.

TYPICAL ANALYSES.

APPENDIX A.

ANALYSES OF WATERS FROM VARIOUS SOURCES.

	1 Burton Well Water.	2 Suitable Stout Water.	3 Suitable Mild-ale Water.	4 Loch Katrine Water.	5	6	7	8	9
Total solids dried at 212° F. (100° C.)	138·92	21·97	67·02	4·15	31·20	12·08	45·73	66·38	4·80
Saline residue	124·40	21·33	65·62	3·89	30·42	10·70	42·64	64·68	3·96
Organic and volatile matter	14·52	0·64	1·40	0·26	0 78	1·38	3·09	1·70	0·81
Lime	36·33	9·79	13·13	0·54	9·83	3·80	3·45	3·50	0·44
Magnesia	10·15	0·43	1·91	0·24	1·38	0·52	0·80	2·14	0·16
Sulphuric acid	52·29	2·62	3·67	0·23	4·24	1·70	7·98	8·40	0·20
Chlorine	2·37	1·11	23·81	1·40	2·14	1·10	6·40	17·63	1·64
Iron	0·09	0·24	0·24	..	Trace.	..	0·46	..	Trace.
Soda	7·25	0·97	18·62	0·94	2·40	0·93	17·70	27·77	1·39
Potash	0·86	..	..	..	0·33	0·25	..	..	..
Silica	0·84	0·26	0·22	..	..	..	..	..	..
Nitric acid	1·25	..	..	..	2·55	..	..	..	..
Nitrous acid	..	..	..	..	..	..	..	..	..
	Parts per Million.								
Free or saline ammonia	0·122	Nil.	0·035	0·011	0·110	0·198	0·340	0·650	0·012
Albuminoid ammonia	0·062	0·021	0·021	0·009	0·035	0·160	0·055	0·030	0·169
	Parts per 100,000.								
Oxygen required to oxidise organic matter	0·095	0·048	0·083	0·040	0·043	0·159	0·064	0·014	0·254
	Most Probable Combination. (Grains per Gallon.)								
Calcic sulphate	77·82	4·44	6·23	0·39	6·46	2·53	..	..	0·34
,, carbonate	7·66	14·21	16·37	0·67	10·44	4·92	6·16	6·24	0·67
Magnesic sulphate	..	..	..	..	..	..	..	4·49	..
,, carbonate	21·31	0·90	4·01	0·50	2·89	1·09	1·68	..	0·33
,, nitrate	..	..	..	..	..	..	..	..	..
Sodic chloride	3·90	1·82	35·12	2·29	3·95	1·75	10·54	29·05	2·64
Calcic ,,	..	..	3·90	..	..	..	..	..	..
Sodic sulphate	10·26	..	..	..	2·24	..	14·16	14·91	..
Potassic sulphate	1·59	..	..	..	0·61	0·46	..	.	..
Calcic nitrate	..	..	..	..	3·87	..	..	..	..
Sodic ,,	1·96	..	..	..	..	..	..	..	..
,, carbonate	..	..	..	..	..	..	10·15	10·03	..

No. 1.—This is a strong Burton water of fair purity. The high proportion of sulphate of lime and carbonate of magnesia, and the small proportion of carbonate of lime, will be noted. The carbonates would be precipitated on boiling, and all the possible beneficial effects to be derived from the presence of sulphate of lime in a brewing water may be obtained with from 50 to 75 grains per gallon of that salt. The chlorides are very small in quantity.

No. 2.—The distinguishing feature of this water, which is of exceptional purity and an excellent one for stout and porter production, is the small quantity of sulphate of lime and all other constituents, with the exception of calcic carbonate, which is in fair proportion and precipitable on boiling.

No. 3.—The essential characteristic of this water, which is also pure, is the high amount of chlorides and the small amount of calcic sulphate. Such water is excellent for mild-ale production.

No. 4.—Here we have a marked contrast between the previously mentioned waters. It is a soft water and of extreme organic purity. Such waters, however, are usually objectionable for steam boilers, as they generally contain humic and other acids, which have a marked corroding effect upon the boiler plates.

No. 5.—Waters of this type are somewhat objectionable for brewing on account of the nitric acid they contain.

Many such supplies are drawn from wells all over the kingdom, particularly in the west of England and north as far as Edinburgh. Despite the nitrates, however, they are usually excellent for brewing, provided a vigorous supply of yeast is employed for the fermentations.

No. 6.—This is a badly contaminated, town supply water, the defilement being mainly of vegetable origin.

No. 7.—Water drawn from a deep London well. It contains sulphate and carbonate of soda and a large quantity of free ammonia. It is excellent for black beers but unsuitable for pale ales; and even artificial manipulation cannot remedy this drawback, since after such treatment the chlorides are excessive.

No. 8 is a sample drawn from a well in Gloucestershire; it is similar in character to No. 7. It contains a large quantity of free ammonia, but is nevertheless pure.

No. 9 is an extremely soft water, but peaty. Numerous waters of this description are to be found, as drawn from shallow wells, in various parts of the west of England, but particularly in Yorkshire.

COMPOSITION OF BOILER SCALE.

	A.	B.	C.	D.	E.
Calcic carbonate	81·62	32·16	5·45	43·65	0·97
,, hydroxide	...	...	13·70	...	...
,, sulphate	2·50	5·64	1·69	34·78	85·53
Magnesic hydroxide	4·63	...	56·37	4·34	3·39
,, carbonate	...	20·04	7·36	...	...
Sodium salts (chiefly chlorides)	0·37	3·31	...	0·56	2·79
Oxide of iron	2·53	7·46	2·81	3·44	0·32
Silica	3·75	16·94	11·70	7·52	1·10
Organic matter	4·60	7·67	0·89	1·55	...
Moisture		6·78		4·16	5·90

COMPOSITION OF KAINIT.

Potassic sulphate	21·3
Magnesic sulphate	14·5
,, chloride	12·4
Potassic chloride	2·0
Sodic chloride	34·6
Calcic sulphate	1·7
Silica	0·8
Moisture	12·7
	100·0

COMPOSITION OF GYPSUM.

Calcic sulphate		80·12
,, oxide	33·56	
Sulphuric anhydride	46·56	
Moisture		19·88
		100·00

COMPOSITION OF EPSOM SALT.

Magnesic sulphate		49·22
,, oxide	16·94	
Sulphuric anhydride	32·28	
Moisture		50·78
		100·00

COMPOSITION OF CHARCOAL.

	New.	Stock.
Carbon, nitrogenous matter, etc.	9·69	15·98
Calcic phosphate	78·75	75·78
,, carbonate	8·41	5·72
,, sulphate	0·24	0·96
,, sulphide	0·11	0·24
Alkaline salts	1·50	Nil.
Oxide of iron	0·15	0·40
Silica	1·15	0·92

COMPOSITION OF COAL.

	Caermarthenshire.			Pembrokeshire.			Glamorganshire.				
	Gwendraeth.	Pontyeats.	Big Vein.	Bonville Court.	Timber Vein.	Watney's (Lyon Playfair).	Brass Vein, Cwmllynfell.	Cawm Neath.	Ystalyfera.	Swansea.	Neath Abbey.
Carbon	92·17	91·16	88·70	94·18	93·00	92·17	91·44	93·12	92·46	89·00	91·08
Hydrogen	3·10	3·11	} 7·40	2·99	3·08	3·10	3·46	} 5·22	6·04	7·50	5·01
Oxygen	2·22	2·74		0·50	1·67	2·22	2·58				
Nitrogen	1·08	0·91		0·76	0·54	1·08	0·21				
Sulphur	0·34	0·86	0·50	0·59	0·68	0·34	0·79				
Ash	1·09	1·12	3·40	0·98	1·03	1·09	1·52	1·50	1·50	3·50	4·00
Total	100·00	99·90	100·00	100·00	100·00	100·00	100·00	99·84	100·00	100·00	100·09

COMPOSITION OF COKE.

	Coking Coals, Durham.					Cannel, Cumberland.	Steam Coal, Wales.	Scotch Cannel.			
	Hamsteels.	Consett.	Whitworth.	South Brancepeth	Dukinfield, Stalybridge.	Dovenby Colliery, Dearham, Maryport.	Black Vein, Merthyr Tydvil.	Lesmahago.	Boghead.	Capeldrea.	Lochgelly.
Carbon	92·55	91·88	91·56	93·41	85·84	57·65	87·92	79·69	29·50	76·52	69·93
Hydrogen	..	..	..	..	0·52	..	4·37	..	..	..	..
Oxygen	..	..	..	..	} 1·38	..	{ 4·88	..	..	..	..
Nitrogen	..	..	..	..		..	{ 0·51	..	..	..	..
Sulphur	0·81	0·84	1·21	0·91	0·86	2·02	0·90	2·26	0·25	0·41	0·37
Ash	6·36	6·91	6·69	5·30	11·40	40·33	1·08	18·05	70·25	23·07	29·70
Water	0·21	0·37	0·54	0·36	..	..	..	..	..	..	..

COMPOSITION OF BITUMINOUS COALS.

	Hartley, Northumberland.	Brockwell Steam, Brancepeth, Durham.	Silkstone, Yorkshire.	Black Vein, Celynear, Merthyr Tydvil.	
	House.	Coking.	Gas.	Steam.	Peat.
Carbon	84·824	83·40	80·46	85·98	54·1
Hydrogen	5·522	4·40	5·08	4·70	5·6
Oxygen	6·223 }	} 7·18	{ 6·80	5·53	} 40·1
Nitrogen	2·075 }		{ 1·67	0·90	
Sulphur	1·181	1·00	1·65	0·59	...
Ash	0·715	3·50	3·30	2·30	4·6
Water	...	0·99	1·04	...	...

COMPOSITION OF PEAT USED AS FUEL.

Carbon	61·02
Hydrogen	5·87
Oxygen	32·40
Nitrogen	0·81
Mineral matter	7·90

COMPOSITION OF WOOD USED AS FUEL.

	Oak.	Beech.	Pine.
Carbon	48·12	49·46	50·62
Hydrogen	6·06	5·96	6·27
Oxygen	44·43	42·36	42·58
Nitrogen	...	1·22	...
Mineral matter	1·37	1·00	0·53

COMPOSITION OF THE PRINCIPAL CEREALS.

	Wheat.	Barley.	Oats.	Rye.	Maize.	Rice.
Starch	62·3	57·0	56·1	54·9	54·8	78·8
Cellulose	8·3	8·3	1·0	6·4	14·9	0·2
Gum and sugar	3·8	3·0	5·7	11·3	2·9	1·6
Fat and oils	1·2	2·5	4·6	2·0	4·7	0·1
Moisture	11·1	14·0	14·2	14·3	11·5	10·8
Albuminoids (soluble)	2·9	1·0	1·3	3·3	0·5	0·2
,, (insoluble)	8·0	12·2	14·7	5·5	8·4	7·0
Ash	2·4	2·0	2·4	2·3	2·3	1·3
	100·0	100·0	100·0	100·0	100·0	100·0

COMPOSITION OF OATMEAL.

Starch	60·96
Cellulose	6·99
Albuminoids	13·47
Fat and oil	7·30
Ash	1·74
Moisture	9·54
Extract per quarter 78 lbs.	100·00

TYPICAL ANALYSIS OF ENGLISH AND FOREIGN MALTS.

	English.		Foreign.	
	1	2	3	4
Specific gravity 10 per cent. wort .	1027·5	1027·3	1025·1	1025·5
Extract per quarter . . .	92·40	91·72	84·33	85·68
Dry extract per cent. . . .	71·24	70·72	65·02	66·06
Saccharification period (minutes) .	20	25	20	16
Colour of wort	5°	4°	5°	3°
Diastatic power	38°	26°	40·2°	34·5°
Acidity of wort	0·11	0·09	0·12	0·13
Mineral matter	1·06	1·08	2·30	2·10
Total albuminoids	10·20	9·10	8·40	9·20
Soluble ,, ...	2·10	2·14	1·90	1·76
Insoluble ,, ...	8·10	6·96	6·50	7·44
Ready-formed soluble carbohydrates	16·51	17·72	11·13	13·12
Moisture	1·10	0·52	1·96	1·20
Arsenic trioxide, grains per lb. .	$\frac{1}{200}$	$\frac{1}{100}$	$\frac{1}{50}$	Free

Biological Examination.

		Hours of Standing.			
		24	30	36	42
1.	Wort filtered from mash . .	Sound.	Sound.	Sound.	Putrid.
	Mash and wort together . .	,,	Cloudy.	Putrid.	...
2.	Wort filtered from mash . .	,,	Sound.	Sound.	Cloudy.
	Mash and wort together . .	,,	,,	Cloudy.	Putrid.
3.	Wort filtered from mash . .	,,	,,	Sound.	Cloudy.
	Mash and wort together . .	,,	Cloudy.	Putrid.	...
4.	Wort filtered from mash . .	,,	Sound.	Cloudy.	Putrid.
	Mash and wort together . .	,,	,,	,,	,,

TYPICAL ANALYSIS OF ENGLISH HIGH-DRIED AND PALE MALTS.

	High Dried.	Pale.
Specific gravity 10 per cent. wort	1027·80	10285·0
Extract per quarter	93·40	95·76
Dry extract per cent.	72·02	73·83
Diastatic power	31·00	44·20
Colour of wort	7·5°	4·0°
Analysis calculated on 100 Grams of Malt.		
Dextrin	17·37	19·71
Maltose	35·36	35·91
Ready-formed soluble carbohydrates	14·12	13·11
Mineral matter	1·76	1·64
Soluble albuminoids	2·32	2·33
Acidity	0·27	0·23
Moisture	1·23	1·78
Grains	27·57	25·29

COMPOSITION OF MALT DUST.

Moisture	1·86
Mineral matter	7·63
Albuminoids	41·23

COMPOSITION OF BARLEY AND MALT.

	Barley.	Malt.
Starch	47·0	46·0
Sugars	3·0	16·0
Fat	2·5	2·5
Albuminoids (soluble)	1·0 } 13·2	3·8 } 12·5
,, (insoluble)	12·2	8·7
Cellular matter (digestible fibre)	14·0	13·5
Cellulose (woody fibre)	4·3	4·7
Ash	2·0	2·3
Moisture	14·0	2·5
	100·0	100·0

COMPOSITION OF MAIZE AND RICE GRITS.

	Maize Grits.	Rice Grits.
Starch	74·00	79·50
Oil	0·94	0·79
Albuminous matter	9·00	8·93
Ash.	0·40	0·28
Moisture	10·61	9·82
Cellulose, etc.	5·05	0·68
	100·00	100·00

COMPOSITION OF FLAKED RICE AND MAIZE.

	Flaked Maize.	Flaked Rice.
Starch	80·70	81·75
Oil	1·20	0·30
Albuminous matter	9·69	8·62
Ash.	0·46	0·34
Moisture	6·32	7·80
Cellulose, etc.	1·63	1·19
	100·00	100·00

SOLUBLE NITROGENOUS CONSTITUENTS OF BARLEY AND OF THE MALT MADE FROM IT, CALCULATED ON THE DRY SUBSTANCE.

	Nitrogen of Albumin.	Nitrogen of Peptone.	Nitrogen of Ammonium Salts.	Nitrogen of Amido Acids.	Nitrogen of Amides.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Barley . .	0·0600	0·0046	0·0169	0·0417	...
Barley steeped .	0·0354	0·0009	...	0·0294	...
Green malt .	0·1671	0·0058	0·0290	0·1417	0·0505
Dried malt .	0·1194	0·0233	0·0057	0·2257	0·0029

COMPOSITION OF ENZYMES.

	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Ash.
Malt diastase . . .	45·68	6·90	4·57	...	6·08
,, ,, ...	47·57	6·49	5·14	...	3·16
Yeast invertase . .	43·10	7·80	4·30	...	6·10
,, ,, . .	43·90	8·40	6·00	0·63	...
,, ,, . .	40·50	6·90	9·30	...	...
Ptyalin	43·10	7·80	11·86	...	6·10
Trypsin	52·75	7·50	16·55	...	17·70
Pepsin	53·20	6·70	17·80	...	...
Pancreatin . . .	43·60	6·50	13·81	0·88	17·04
Emulsin . . .	43·06	7·20	4·52	1·25	...
,, . . .	48·80	7·10	14·20	1·30	...

GELATINISATION TEMPERATURES OF VARIOUS STARCHES.

Variety of Starch.	Temperature of Gelatinisation.	
	°F.	°C.
Potato . . .	149	65
Rice	158–167	70–75
Barley . . .	176	80
Green malt . .	185	85
Kilned ,, . .	176	80
Wheat . . .	167–176	75–80
Maize . . .	158–167	70–75
Rye	176	80
Oats	185	85

COMPOSITION OF RAW SUGARS AND SYRUPS.

	Raw Jaggery.	Partially Refined Jaggery.	Raw Penang.	Raw Egyptian.	Molasses.	"Green" Syrup.	Treacle.
Cane-sugar	75·12	90·94	76·40	80·34	48·10	51·33	34·40
Invert-sugar	11·03	3·31	11·59	4·32	17·90	15·50	26·29
Other organic matters	3·14	0·73	2·34	0·82	1·50	12·37	17·20
Ash	5·33	0·63	2·89	7·33	1·30	3·70	4·91
Moisture	5·38	4·39	6·78	7·19	31·20	17·10	17·20
	100·00	100·00	100·00	100·00	100·00	100·00	100·00

COMPOSITION OF VARIOUS QUALITIES OF INVERT SUGAR.

	A.	B.	C.	D.
Specific gravity 10 per cent. solution	1032·45	1031·50	1032·40	1032·55
Extract per cwt.	36·34	35·28	36·28	36·45
Extract per cent. (allowance made for ash)	82·46	78·93	80·19	80·37
Invert-sugar	77·76	71·94	71·38	71·23
Cane-sugar	1·50	2·00	2·70	3·00
Albuminoids	...	·80	·79	·89
Moisture	17·54	21·07	19·81	19·63
Mineral matter	1·50	2·50	3·50	3·70
Unfermentable bodies	1·70	1·69	1·82	1·55
Percentage Composition of Dry Extract.				
Invert-sugar	94·30	91·14	89·02	88·63
Cane-sugar	1·82	2·53	3·36	3·74
Albuminoids	...	1·01	·98	1·10
Mineral matter	1·82	3·17	4·37	4·60
Unfermentable bodies	2·06	2·15	2·27	1·93
	100·00	100·00	100·00	100·00

COMPOSITION OF PRIMING SYRUP.

Specific gravity of 20 per cent. solution	1048·71
Extract per cwt.	27·27
Extract per cent. (allowance made for ash)	61·27
Invert-sugar	55·79
Cane-sugar	1·38
Albuminoids	·93
Moisture	38·73
Mineral matter	1·67
Unfermentable bodies	1·50

Percentage Composition of Dry Extract.

Invert-sugar	91·06
Cane-sugar	2·26
Albuminoids	1·51
Mineral matter	2·72
Unfermentable bodies	2·45
	100·00

COMPOSITION OF VARIOUS QUALITIES OF GLUCOSE.

	A.	B.	C.	D.
Specific gravity of 10 per cent. solution	1035·00	1035·10	1033·00	1032·76
Extract per cwt.	39·20	39·31	36·96	36·68
Extract per cent. (allowance made for ash)	89·28	90·72	83·78	83·70
Dextrose	75·28	73·34	61·67	65·92
Maltose	...	2·10	6·12	2·13
Dextrin	1·00	1·15	1·25	1·30
Albuminoids	·20	·45	·60	·57
Moisture	10·72	9·28	16·22	16·30
Mineral matter	1·30	0·20	1·60	1·10
Unfermentable bodies	11·50	13·48	12·54	12·68
Percentage Composition of Dry Extract.				
Dextrose	84·31	80·85	73·61	78·75
Maltose	...	2·32	7·30	2·54
Dextrin	1·12	1·26	1·49	1·56
Albuminoids	·22	·41	·72	·68
Mineral matter	1·46	·32	1·92	1·32
Unfermentable bodies	12·89	14·85	14·96	15·15
	100·00	100·00	100·00	100·00

COMPOSITION OF DEXTRINOUS CARAMEL.

Specific gravity of 20 per cent. solution	1076·80
Extract per cwt.	43·00
Extract per cent. (allowance made for ash)	98·21
Dextrin	61·94
Dextrose	16·44
Albuminoids	·19
Moisture	1·79
Mineral matter	1·16
Unfermentable bodies	18·48

Percentage Composition of Dry Extract.

Dextrin	63·07
Dextrose	16·73
Albuminoids	·19
Mineral matter	1·19
Unfermentable bodies	18·82
	100·00

COMPOSITION OF FLUID CARAMEL.

Specific gravity of 20 per cent. solution	1059·26
Extract per cwt.	33·18
Extract per cent. (allowance made for ash)	74·86
Dextrose	57·50
Albuminoids	·28
Moisture	25·14
Mineral matter	1·78
Unfermentable bodies	15·30

Percentage Composition of Dry Extract.

Dextrose	76·81
Albuminoids	·37
Mineral matter	2·38
Unfermentable bodies	20·44
	100·00

COMPOSITION OF YEAST.

	Nageli and Löw.	Belohoubek.	Average.
Cellulose	37·0	49·6	43·3
Albumin	47·0	41·0	44·0
Fat	5·0	2·8	3·9
Extractive matter	4·0	1·1	2·5
Ash	7·0	5·5	6·3
	100·0	100·0	100·0

COMPOSITION OF THE ASH OF YEAST.

	Yeast from Hard Burton Water.	Yeast from Soft Water.
Potassium phosphate	63·1	93·9
Magnesium ,,	21·0	5·6
Calcium ,,	13·6	traces
Silica, alumina, etc.	2·3	0·5
	100·0	100·0

DECOMPOSITION OF SUGAR DURING FERMENTATION.

(Pasteur.)

100 parts of dextrose yield:—

Alcohol	48·3
Carbonic acid gas	46·4
Glycerol	2·5 to 3·6
Succinic acid	0·4 to 0·7
Cellulose and other bodies	1·3

COMPOSITION OF WET AND DRIED BREWERS' GRAINS.

(J. O'Sullivan.)

	Wet Grains.	Dried Grains.
Water	75·0	7·5
Fat	1·2	3·8
Starch, sugar, and other carbohydrates	1·3	5·0
Albuminoids	4·7	19·1
Cellular matter (digestible fibre)	11·8	40·2
Cellulose (woody fibre)	5·0	19·6
Ash	1·0	4·8
	100·0	100·0

COMPOSITION OF BREWERS' GRAINS COMPARED WITH THAT OF SWEDE TURNIPS AND MANGOLDS.

(J. O'Sullivan.)

	Brewers' Wet Grains.	Swede Turnips.	Mangolds.
Water	75·0	89·3	88·5
Fat	1·2	0·2	0·1
Starch, sugar, and other carbohydrates	1·3	7·3	8·2
Albuminoids	4·7	2·2	1·6
Cellular matter (digestible fibre)	11·8	1·1	1·0
Cellulose (woody fibre)	5·0		
Ash	1·0	0·6	1·0
Total food constituents	19·0	10·8	10·9

COMPOSITION OF MALT ROOTLETS.

Starch, sugar, and mucilage	30·70
*Nitrogenous matter	29·50
Woody fibre	2·90
Ash	3·30
Fat	32·90
Moisture	0·70
	100·00

* This figure is very large, and varies with different samples.

COMPOSITION OF BISULPHITE OF LIME: 100 PARTS BY VOLUME AND BY WEIGHT.

	Weight.	Volume.
Specific gravity	106·9 grams.	1069·0
Total sulphurous acid	6·52	6·97
Free ,, ,,	3·83	4·10
Combined ,, ,,	2·68	2·87
Calcic sulphate	0·31	0·34
,, sulphite	4·62	4·94
Magnesic sulphite	0·36	0·39
Sodic chloride	0·008	0·009
Iron oxide	0·003	0·004
Hyposulphites	Nil.	Nil.

COMPOSITION OF KALIUM METASULPHITE.

Moisture at 212° F. (100° C.)	1·48
Loss by ignition	20·49
Potassium	42·73
Sulphuric acid	2·12
Sulphurous acid	55·48

Most Probable Combination.

Moisture	1·48
Potassic sulphate	4·61
,, sulphite	68·25
Free sulphurous acid	27·84

COMPOSITION OF MONOSULPHITE OF LIME.

Lime	43·79
Magnesia	0·57
Sulphuric acid	1·82
Sulphurous acid	41·29
Chlorine	0·32
Iron and alumina	1·97
Silica	0·16

Most Probable Combination.

Calcic sulphite	77·41
,, sulphate	3·09
Magnesic sulphite	0·91
Calcic oxide	6·40
Sodic chloride	0·52
Iron and alumina	1·97
Silica	0·16
Moisture, carbon dioxide, etc., by difference	9·54
	100·00

COMPOSITION OF POTASSIC CARBONATE.

Total alkalinity	85·94
Potassic chloride	2·92
,, sulphate	0·66
Moisture	10·42

COMPOSITION OF CAUSTIC POTASH.

Total alkalinity	87·92
Potassic chloride	6·54
,, sulphate	1·43
Moisture	0·79
Carbon dioxide, traces of iron, etc.	3·22

COMPOSITION OF CAUSTIC SODA.

Total alkalinity	96·83
Sodic chloride	1·72
,, sulphate	·58
Moisture	·89

APPENDIX B.

TABLES AND FACTORS.

Multipliers required in Volumetric Analysis.

Normal sulphuric acid, H_2SO_4 . .	1 c.c. $= 0 \cdot 049$	gram	H_2SO_4.
	,, $= 0 \cdot 048$	,,	SO_4.
	,, $= 0 \cdot 040$	,,	SO_3.
	,, $= 0 \cdot 053$	,,	Na_2CO_3.
	,, $= 0 \cdot 090$	,,	albumin.
Normal hydrochloric acid, HCl . .	1 c.c. $= 0 \cdot 0365$	,,	HCl.
	,, $= 0 \cdot 0355$	,,	Cl.
Normal nitric acid, HNO_3 . .	1 c.c. $= 0 \cdot 063$	,,	HNO_3.
	,, $= 0 \cdot 062$	,,	NO_3.
	,, $= 0 \cdot 054$	,,	N_2O_5.
Normal oxalic acid, $H_2C_2O_4$ . .	1 c.c. $= 0 \cdot 063$	,,	$H_2C_2O_4$, 2OH
	,, $= 0 \cdot 045$	,,	$H_2C_2O_4$.
Normal sodium hydrate, NaHO .	1 c.c. $= 0 \cdot 040$	,,	NaHO.
	,, $= 0 \cdot 031$	,,	Na_2O.
Normal potassium hydrate, KHO .	1 c.c. $= 0 \cdot 056$	,,	KHO.
	,, $= 0 \cdot 047$	,,	K_2O.
Normal sodium carbonate, Na_2CO_3 .	1 c.c. $= 0 \cdot 053$	,,	Na_2CO_3.
	,, $= 0 \cdot 030$	,,	CO_3.
	,, $= 0 \cdot 022$	,,	CO_2.

Calcium (Ca = 40)—

1 c.c. permanganate	$= 0 \cdot 0028$	,,	CaO.
,, ,,	$= 0 \cdot 0050$	,,	$CaCO_3$.
,, ,,	$= 0 \cdot 0086$	,,	$CaSO_4$, $2OH_2$.
1 c.c. normal oxalic acid	$= 0 \cdot 0280$	,,	CaO.

Cryst. oxalic acid $\times 0 \cdot 444$ = CaO.
Double iron salt $\times 0 \cdot 07143$ = CaO.

Chlorine (Cl = 35·37)—

1 c.c. $\frac{N}{10}$ silver solution	$= 0 \cdot 003537$	gram	Cl.
,, ,, ,,	$= 0 \cdot 005837$	,,	NaCl.
1 c.c. $\frac{N}{10}$ arsenious or hyposulphite solution	$= 0 \cdot 003537$	,,	Cl.

1 litre of chlorine at 0° C. and 760 mm. weighs 3·17 grams.

Chromium ($Cr = 52 \cdot 4$)—

Metallic iron	$\times 0 \cdot 3123 =$	Cr.
,, ,,	$\times 0 \cdot 5981 =$	CrO_3.
,, ,,	$\times 0 \cdot 8784 =$	$K_2Cr_2O_7$.
,, ,,	$\times 1 \cdot 926 =$	$PbCrO_4$.
Double iron salt	$\times 0 \cdot 0446 =$	Cr.
,, ,,	$\times 0 \cdot 0854 =$	CrO_3.
,, ,,	$\times 0 \cdot 1255 =$	$K_2Cr_2O_7$.
,, ,,	$\times 0 \cdot 0275 =$	$PbCrO_4$.

1 c.c. $\frac{N}{10}$ solution $= 0 \cdot 003349$ gram CrO_3.

,, ,, $= 0 \cdot 00492$,, $K_2Cr_2O_7$.

Copper ($Cu = 63$)—

1 c.c. $\frac{N}{10}$ solution $= 0 \cdot 0063$ gram Cu.

Iron $\times 1 \cdot 125 =$ copper.
Double iron salt $\times 0 \cdot 1607 =$,,

Cyanogen ($CN = 26$)—

1 c.c. $\frac{N}{10}$ silver solution $= 0 \cdot 0052$ gram CN.
,, ,, ,, $= 0 \cdot 0054$,, HCN.
,, ,, ,, $= 0 \cdot 01302$,, KCN.
1 c.c. $\frac{N}{10}$ iodine solution $= 0 \cdot 003255$,, KCN.

Gold ($Au = 196 \cdot 5$)—

1 c.c. normal oxalic acid $= 0 \cdot 0655$ gram Au.

Iodine ($I = 126 \cdot 5$)—

1 c.c. $\frac{N}{10}$ hyposulphite $= 0 \cdot 01265$ gram iodine.

1 c.c. $\frac{N}{10}$ iodine $= 0 \cdot 0032$,, SO_2.

Iron ($Fe = 56$)—

1 c.c. $\frac{N}{10}$ permanganate, bichromate, or hyposulphite $= 0 \cdot 0056$ Fe.
,, ,, ,, ,, $= 0 \cdot 0072$ FeO.
,, ,, ,, ,, $= 0 \cdot 0017$ H_2S.
,, ,, ,, ,, $= 0 \cdot 0056$ CaO.

Lead ($Pb = 206 \cdot 4$)—

1 c.c. $\frac{N}{10}$ permanganate $= 0 \cdot 01032$ gram Pb.

1 c.c. normal oxalic acid $= 0 \cdot 1032$,, Pb.
Metallic iron $\times 1 \cdot 842 =$ Pb.
Double iron salt $\times 0 \cdot 263 =$ Pb.

Manganese ($Mn = 55$)—

$MnO = 71$. $MnO_2 = 87$.

Metallic iron	$\times 0 \cdot 491 =$	Mn.
,, ,,	$\times 0 \cdot 63393 =$	MnO.
,, ,,	$\times 0 \cdot 7768 =$	MnO_2.
Double iron salt	$\times 0 \cdot 0911 =$	MnO.
,, ,,	$\times 0 \cdot 111 =$	MNO_2.
Cryst. oxalic acid	$\times 0 \cdot 6916 =$	MnO_2.

1 c.c. $\frac{N}{10}$ solution $= 0 \cdot 00355$ gram MnO.

,, ,, $\times 0 \cdot 00435$,, MnO_2.

Mercury ($Hg = 200$)—

Double iron salt $\times 0\cdot5104 = Hg$.
,, ,, $\times 0\cdot6914 = HgCl_2$.
1 c.c. $\frac{N}{10}$ solution $= 0\cdot0200$ gram Hg.
,, ,, $= 0\cdot0208$,, Hg_2O.
,, ,, $= 0\cdot0271$,, $HgCl_2$.

Nitrogen as nitrates and nitrites—

$N_2O_5 = 108$. $N_2O_3 = 76$.
Normal acid $\times 0\cdot0540 = N_2O_5$.
,, ,, $\times 0\cdot1011 = KNO_3$.
Metallic iron $\times 0\cdot3750 = HNO_3$.
,, ,, $\times 0\cdot6018 = KNO_3$.
,, ,, $\times 0\cdot3214 = N_2O_5$.

Potassic ferrocyanide (K_4FeCy_6, $3OH_2 = 422$)—

Metallic iron $\times 7\cdot541 =$ cryst. potassic ferrocyanide.
Double iron salt $\times 1\cdot077 =$,, ,,

Potassic ferricyanide ($K_6Fe_2Cy_{12} = 658$)—

Metallic iron $\times 5\cdot88 =$ potassic ferricyanide.
Double iron salt $\times 1\cdot68 =$,, ,,
$\frac{N}{10}$ hyposulphite $\times 0\cdot0329 =$,, ,,

Silver ($Ag = 107\cdot66$)—

1 c.c. $\frac{N}{10}$ $NaCl = 0\cdot010766$ gram Ag.
,, ,, $= 0\cdot016966$,, $AgNO_3$.

Sulphuretted hydrogen ($H_2S = 34$)—

1 c.c. $\frac{N}{10}$ arsenious solution $= 0\cdot00255$ gram H_2S.

Tin ($Sn = 118$)—

Metallic iron $\times 1\cdot0536 = Sn$.
Double iron salt $\times 0\cdot1505 = Sn$.
Factor for $\frac{N}{10}$ iodine or permanganate solution $0\cdot0059$.

Zinc ($Zn = 65$)—

Metallic iron $\times 0\cdot5809 = Zn$.
,, ,, $\times 0\cdot724 = ZnO$.
Double iron salt $\times 0\cdot08298 = Zn$.
,, ,, $\times 0\cdot1034 = ZnO$.
1 c.c. $\frac{N}{10}$ solution $= 0\cdot00325$ gram Zn.

TABLE SHOWING EXTRACT IN POUNDS PER QUARTER AND DRY EXTRACT PER CENT. OBTAINED FROM PALE OR COLOURED MALT OR MALT AND RAW GRAIN, MASHED TO OBTAIN A 10 PER CENT. SOLUTION.

Example.—Specific gravity 10 per cent. solution less 1000 × 3·36 = lbs. extract per quarter.
" " " " " × ·36 = lbs. per barrel.
" " " " " ÷ 3·86 = dry extract per cent.

(BAILEY.)

Specific Gravity.	Lbs. per Barrel.	Extract per Quarter.	Dry Extract per Cent.	Specific Gravity.	Lbs. per Barrel.	Extract per Quarter.	Dry Extract per Cent.
1017·0	6·12	57·12	44·04	1021·0	7·56	70·56	54·40
·1	6·15	57·75	44·30	·1	7·59	70·89	54·66
·2	6·19	57·79	44·55	·2	7·63	71·23	54·92
·3	6·22	58·12	44·81	·3	7·66	71·56	55·18
·4	6·26	58·26	45·07	·4	7·70	71·90	55·44
·5	6·30	58·80	45·33	·5	7·74	72·24	55·69
·6	6·33	59·13	45·59	·6	7·77	72·57	55·95
·7	6·37	59·47	45·85	·7	7·81	72·91	56·21
·8	6·40	59·80	46·11	·8	7·84	72·58	56·47
·9	6·44	60·14	46·37	·9	7·88	73·58	56·73
1018·0	6·48	60·48	46·63	1022·0	7·92	73·92	56·99
·1	6·51	60·81	46·89	·1	7·98	74·25	57·25
·2	6·55	61·15	47·15	·2	7·99	74·59	57·51
·3	6·58	61·48	47·40	·3	8·02	74·92	57·77
·4	6·62	61·82	47·66	·4	8·06	75·26	58·03
·5	6·66	62·16	47·92	·5	8·10	75·60	58·29
·6	6·69	62·49	48·18	·6	8·13	75·93	58·54
·7	6·73	62·83	48·44	·7	8·17	76·27	58·80
·8	6·76	63·16	48·70	·8	8·20	76·60	59·06
·9	6·80	63·50	48·96	·9	8·24	76·94	59·32
1019·0	6·84	63·84	49·22	1023·0	8·28	77·28	59·58
·1	6·87	64·17	49·48	·1	8·31	77·61	59·84
·2	6·91	64·51	49·74	·2	8·35	77·95	60·10
·3	6·94	64·84	50·00	·3	8·38	78·28	60·36
·4	6·98	65·18	50·25	·4	8·42	78·62	60·62
·5	7·02	65·52	50·51	·5	8·46	78·96	60·88
·6	7·05	65·85	50·77	·6	8·49	79·29	61·13
·7	7·09	66·19	51·03	·7	8·53	79·63	61·39
·8	7·12	66·32	51·29	·8	8·56	79·96	61·65
·9	7·16	66·86	51·55	·9	8·60	80·30	61·91
1020·0	7·20	67·20	51·81	1024·0	8·64	80·64	62·17
·1	7·23	67·53	52·07	·1	8·67	80·97	62·43
·2	7·27	67·87	52·33	·2	8·71	81·31	62·69
·3	7·30	68·20	52·59	·3	8·74	81·64	62·95
·4	7·34	68·54	52·84	·4	8·78	81·98	63·21
·5	7·38	68·88	53·10	·5	8·82	82·32	63·47
·6	7·41	69·21	53·36	·6	8·85	82·65	63·73
·7	7·45	69·55	53·62	·7	8·89	82·99	63·98
·8	7·48	69·88	53·88	·8	8·92	83·32	64·24
·9	7·52	70·02	54·14	·9	8·96	83·66	64·50

TABLE SHOWING EXTRACT IN POUNDS PER QUARTER, ETC.—*continued.*

Specific Gravity.	Lbs. per Barrel.	Extract per Quarter.	Dry Extract per Cent.	Specific Gravity.	Lbs. per Barrel.	Extract per Quarter.	Dry Extract per Cent.
1025·0	9·00	84·00	64·76	1029·0	10·44	97·44	75·12
·1	9·03	84·33	65·02	·1	10·47	97·77	75·38
·2	9·07	84·67	65·28	·2	10·51	98·11	75·64
·3	9·10	85·00	65·54	·3	10·54	98·44	75·90
·4	9·14	85·34	65·80	·4	10·58	98·78	76·16
·5	9·18	85·68	66·06	·5	10·62	99·12	76·42
·6	9·21	86·01	66·32	·6	10·65	99·45	76·68
·7	9·25	86·35	66·58	·7	10·69	99·79	76·94
·8	9·28	86·68	66·83	·8	10·72	100·12	77·20
·9	9·32	87·02	67·09	·9	10·76	100·46	77·46
1026·0	9·36	87·36	67·35	1030·0	10·80	100·80	77·72
·1	9·39	87·69	67·61	·1	10·83	101·13	78·00
·2	9·43	88·05	67·87	·2	10·87	101·47	78·26
·3	9·46	88·36	68·13	·3	10·90	101·80	78·52
·4	9·50	88·70	68·39	·4	10·94	102·14	78·78
·5	9·54	89·04	68·65	·5	10·98	102·48	79·01
·6	9·57	89·37	68·91	·6	11·01	102·81	79·27
·7	9·61	89·71	69·17	·7	11·05	103·15	79·53
·8	9·64	90·04	69·43	·8	11·08	103·48	79·79
·9	9·68	90·38	69·68	·9	11·12	103·82	80·05
1027·0	9·72	90·72	69·94	1031·0	11·16	104·16	80·31
·1	9·75	91·05	70·20	·1	11·19	104·46	80·56
·2	9·79	91·39	70·46	·2	11·23	104·83	80·82
·3	9·82	91·72	70·72	·3	11·26	105·16	81·08
·4	9·86	92·06	70·98	·4	11·30	105·50	81·34
·5	9·90	92·40	71·24	·5	11·34	105·84	81·60
·6	9·93	92·73	71·50	·6	11·37	106·17	81·86
·7	9·97	93·07	71·76	·7	11·41	106·51	82·12
·8	10·00	93·40	72·02	·8	11·44	106·84	82·38
·9	10·04	93·74	72·27	·9	11·48	107·18	82·64
1028·0	10·08	94·08	72·53	1032·0	11·52	107·52	82·90
·1	10·11	94·41	72·79	·1	11·55	107·85	83·16
·2	10·15	94·75	73·05	·2	11·59	108·19	83·41
·3	10·18	95·08	73·31	·3	11·62	108·52	83·68
·4	10·22	95·42	73·57	·4	11·64	108·86	83·93
·5	10·26	95·76	73·83	·5	11·70	109·20	84·09
·6	10·29	96·09	74·09	·6	11·73	109·54	84·45
·7	10·33	96·43	74·35	·7	11·77	109·87	84·71
·8	10·36	96·76	74·61	·8	11·80	110·20	84·97
·9	10·40	97·10	74·87	·9	11·84	110·54	85·23
				1033·0	11·88	110·88	85·49

Table showing Percentage Matters soluble in Cold Water from which may be calculated the Ready-formed Soluble Carbohydrates. (Malt Analysis—Bailey.)

Example.—Specific gravity of cold-water extract=1005·00. 1005·00 − 1000·00 = 5·00 ÷ 3·86 = 1·209 dry solids per 100 c.c. 1·209 × 10 (10 per cent. solution) = 12·09 per cent.

Deduct percentage of albuminoids, ash and acid, as found by analysis; or, taking these as equal to 4 per cent., the balance equals ready-formed soluble carbohydrates.

Specific Gravity.	Dry Solids per 100 c.c.	Matters soluble in Cold Water.	Specific Gravity.	Dry Solids per 100 c.c.	Matters soluble in Cold Water.
1001·0	0·25	2·50	·6	1·450	14·50
·1	0·28	2·80	·7	1·476	14·76
·2	0·31	3·10	·8	1·502	15·02
·3	0·33	3·30	·9	1·528	15·28
·4	0·36	3·60	1006·0	1·552	15·52
·5	0·38	3·80	·1	1·580	15·80
·6	0·41	4·10	·2	1·606	16·06
·7	0·44	4·40	·3	1·632	16·32
·8	0·46	4·60	·4	1·658	16·58
·9	0·49	4·90	·5	1·683	16·83
1002·0	0·51	5·10	·6	1·709	17·09
·1	0·54	5·40	·7	1·735	17·35
·2	0·56	5·60	·8	1·761	17·61
·3	0·59	5·90	·9	1·787	17·87
·4	0·62	6·20	1007·0	1·813	18·13
·5	0·64	6·40	·1	1·839	18·39
·6	0·67	6·70	·2	1·865	18·65
·7	0·69	6·90	·3	1·891	18·91
·8	0·72	7·20	·4	1·917	19·17
·9	0·75	7·50	·5	1·943	19·43
1003·0	0·77	7·70	·6	1·968	19·68
·1	0·79	7·90	·7	1·994	19·94
·2	0·82	8·20	·8	2·020	20·20
·3	0·85	8·50	·9	2·046	20·46
·4	0·88	8·80	1008·0	2·072	20·72
·5	0·90	9·00	·1	2·098	20·98
·6	0·93	9·30	·2	2·124	21·24
·7	0·95	9·50	·3	2·150	21·50
·8	0·98	9·80	·4	2·176	21·76
·9	1·01	10·10	·5	2·202	22·02
1004·0	1·03	10·30	·6	2·228	22·28
·1	1·06	10·60	·7	2·253	22·53
·2	1·08	10·80	·8	2·279	22·79
·3	1·11	11·10	·9	2·305	23·05
·4	1·14	11·40	1009·0	2·331	23·31
·5	1·16	11·60	·1	2·357	23·57
·6	1·19	11·90	·2	2·383	23·83
·7	1·21	12·10	·3	2·409	24·09
·8	1·24	12·40	·4	2·435	24·35
·9	1·26	12·60	·5	2·461	24·61
1005·0	1·295	12·95	·6	2·487	24·87
·1	1·321	13·21	·7	2·512	25·12
·2	1·344	13·44	·8	2·538	25·38
·3	1·373	13·73	·9	2·564	25·64
·4	1·398	13·98	1010·0	2·590	25·90
·5	1·424	14·24			

REDUCING VALUES OF VARYING QUANTITIES OF DEXTROSE, LEVULOSE, AND INVERT-SUGAR UNDER STANDARD CONDITIONS. (From Brown, Morris, and Millar, *Journal of the Chemical Society*, 1897, vol. lxxi. p. 281.)

Dextrose.				Levulose.				Invert-Sugar.		
Dextrose. Grams.	Cu. Grams.	CuO. Grams.	Cu. Corresponding to 1 Gram Dextrose.	Levulose. Grams.	Cu. Grams.	CuO. Grams.	Cu. Corresponding to 1 Gram Levulose.	Invert-Sugar. Grams.	Cu. Grams.	CuO. Grams.
·050	·1030	·1289	2·060	·050	·0923	·1155	1·846	·050	·0975	·1221
·055	·1134	·1422	2·061	·055	·1027	·1287	1·858	·055	·1076	·1349
·060	·1238	·1552	2·063	·060	·1122	·1407	1·870	·060	·1176	·1474
·065	·1342	·1682	2·062	·065	·1216	·1524	1·871	·065	·1275	·1598
·070	·1443	·1809	2·061	·070	·1312	·1645	1·874	·070	·1373	·1721
·075	·1543	·1935	2·058	·075	·1405	·1761	1·873	·075	·1468	·1840
·080	·1644	·2061	2·055	·080	·1500	·1881	1·875	·080	·1566	·1963
·085	·1740	·2187	2·046	·085	·1590	·1993	1·871	·085	·1662	·2084
·090	·1834	·2299	2·038	·090	·1686	·2114	1·873	·090	·1755	·2200
·095	·1930	·2420	2·033	·095	·1774	·2224	1·868	·095	·1848	·2317
·100	·2027	·2538	2·027	·100	·1862	·2331	1·862	·100	·1941	·2430
·105	·2123	·2662	2·024	·105	·1952	·2447	1·859	·105	·2034	·2550
·110	·2218	·2781	2·020	·110	·2040	·2558	1·855	·110	·2128	·2668
·115	·2313	·2900	2·012	·115	·2129	·2669	1·851	·115	·2220	·2783
·120	·2404	·3014	2·003	·120	·2215	·2777	1·846	·120	·2311	·2898
·125	·2496	·3130	1·997	·125	·2303	·2887	1·843	·125	·2400	·3009
·130	·2585	·3241	1·990	·130	·2390	·2997	1·840	·130	·2489	·3121
·135	·2675	·3354	1·981	·135	·2477	·3106	1·834	·135	·2578	·3232
·140	·2762	·3463	1·973	·140	·2559	·3209	1·828	·140	·2663	·3339
·145	·2850	·3573	1·964	·145	·2641	·3311	1·822	·145	·2750	·3448
·150	·2934	·3673	1·956	·150	·2723	·3409	1·815	·150	·2832	·3546
·155	·3020	·3787	1·948	·155	·2805	·3517	1·811	·155	·2915	·3655
·160	·3103	·3891	1·940	·160	·2889	·3622	1·806	·160	·3002	·3764
·165	·3187	·3996	1·931	·165	·2972	·3726	1·803	·165	·3086	·3869
·170	·3268	·4098	1·922	·170	·3053	·3828	1·799	·170	·3167	·3971
·175	·3350	·4200	1·914	·175	·3134	·3930	1·793	·175	·3251	·4076
·180	·3431	·4302	1·906	·180	·3216	·4032	1·787	·180	·3331	·4177
·185	·3508	·4399	1·896	·185	·3297	·4134	1·782	·185	·3410	·4276
190	·3590	·4501	1·890	·190	·3377	·4234	1·777	·190	·3490	·4376
·195	·3668	·4599	1·881	·195	·3457	·4335	1·773	·195	·3570	·4476
·200	·3745	·4689	1·872	·200	·3539	·4431	1·769	·200	·3650	·4570
·205	·3822	·4792	1·863	·205	·3616	·4534	1·765	·205	·3726	·4672

The sugar values for weights of Cu or CuO lying between any of the weights given in the above table must be arrived at by calculation.

Example.—The amount of dextrose corresponding with ·2385 gram Cu is required. On referring to the table, ·2313 gram Cu corresponds with ·115 gram dextrose, and ·2404 gram Cu with ·120 gram dextrose. Hence ·2404 – ·2313 = ·0091 gram Cu and ·120 – ·115 = ·005 gram dextrose. Therefore ·0091 gram Cu = ·005 gram dextrose in the portion of the table used. Now the difference between the amount of Cu found, ·2385, and the nearest lower amount in the table, ·2313 gram, is ·0072 gram. Hence ·0091 : ·005 : : ·0072 : ·004. Therefore ·115 + ·004 = ·119 gram dextrose, corresponding to ·2385 gram Cu.

Reducing Values of Varying Quantities of Maltose under Standard Conditions. (From Brown, Morris, and Millar, *Journal of the Chemical Society*, 1897, vol. lxxi. p. 100.)

Maltose. Grams.	Cu. Grams.	CuO. Grams.	Cu. Corresponding to 1 Gram Maltose.	Maltose. Grams.	Cu. Grams.	CuO. Grams.	Cu. Corresponding to 1 Gram Maltose.
·070	·0772	·0966	1·1029	·190	·2072	·2593	1·0953
·075	·0826	·1034	1·1026	·195	·2126	·2661	1·0949
·080	·0880	·1102	1·1023	·200	·2180	·2729	1·0946
·085	·0934	·1169	1·1020	·205	·2234	·2797	1·0943
·090	·0988	·1237	1·1017	·210	·2288	·2865	1·0940
·095	·1042	·1305	1·1013	·215	·2342	·2933	1·0937
·100	·1097	·1373	1·1010	·220	·2397	·3000	1·0933
·105	·1151	·1441	1·1007	·225	·2451	·3068	1·0930
·110	·1205	·1509	1·1004	·230	·2505	·3136	1·0927
·115	·1259	·1576	1·1001	·235	·2559	·3203	1·0924
·120	·1313	·1644	1·0997	·240	·2613	·3272	1·0921
·125	·1367	·1712	1·0994	·245	·2667	·3340	1·0917
·130	·1422	·1779	1·0991	·250	·2722	·3407	1·0914
·135	·1476	·1848	1·0988	·255	·2776	·3475	1·0911
·140	·1530	·1916	1·0985	·260	·2830	·3543	1·0908
·145	·1584	·1983	1·0981	·265	·2884	·3610	1·0905
·150	·1634	·2051	1·0978	·270	·2938	·3678	1·0901
·155	·1692	·2119	1·0975	·275	·2992	·3747	1·0898
·160	·1747	·2186	1·0972	·280	·3047	·3814	1·0895
·165	·1801	·2254	1·0969	·285	·3101	·3882	1·0892
·170	·1855	·2323	1·0965	·290	·3155	·3950	1·0889
·175	·1909	·2490	1·0962	·295	·3209	·4017	1·0885
·180	·1963	·2458	1·0959	·300	·3264	·4085	1·0882
·185	·2017	·2526	1·0956	·305	·3318	·4154	1·0879

Otto's Table showing the Percentages of H_2SO_4 corresponding to the Dilute Acid of various Specific Gravities at 15° C.

Per cent. of H_2SO_4.	Specific Gravity.	Per cent. of H_2SO_4.	Specific Gravity.	Per cent. of H_2SO_4.	Specific Gravity.	Per cent. of H_2SO_4.	Specific Gravity.
100	1·8426	75	1·6750	50	1·3980	25	1·1820
99	1·8420	74	1·6630	49	1·3866	24	1·1740
98	1·8406	73	1·6510	48	1·3790	23	1·1670
97	1·8400	72	1·6390	47	1·3700	22	1·1590
96	1·8384	71	1·6270	46	1·3610	21	1·1516
95	1·8376	70	1·6150	45	1·3510	20	1·1440
94	1·8356	69	1·6040	44	1·3420	19	1·1360
93	1·8340	68	1·5920	43	1·3330	18	1·1290
92	1·8310	67	1·5800	42	1·3240	17	1·1210
91	1·8270	66	1·5860	41	1·3150	16	1·1136
90	1·8220	65	1·5570	40	1·3060	15	1·1060
89	1·8160	64	1·5450	39	1·2976	14	1·0980
88	1·8090	63	1·5340	38	1·2890	13	1·0910
87	1·8020	62	1·5230	37	1·2810	12	1·0830
86	1·7940	61	1·5120	36	1·2720	11	1·0756
85	1·7860	60	1·5010	35	1·2640	10	1·0680
84	1·7770	59	1·4900	34	1·2560	9	1·0610
83	1·7670	58	1·4800	33	1·2476	8	1·0536
82	1·7560	57	1·4690	32	1·2390	7	1·0464
81	1·7450	56	1·4586	31	1·2310	6	1·0390
80	1·7340	55	1·4480	30	1·2230	5	1·0320
79	1·7220	54	1·4380	29	1·2150	4	1·0256
78	1·7100	53	1·4280	28	1·2066	3	1·0190
77	1·6980	52	1·4180	27	1·1980	2	1·0130
76	1·6860	51	1·4080	26	1·1900	1	1·0064

Ure's Table showing the Percentages of HCL. corresponding to the Dilute Acid of Various Specific Gravities at 15° C.

Specific Gravity.	Per cent. of HCL.	Per cent. of Acid of 1·20 sp. gr.	Specific Gravity.	Per cent. of HCL.	Per cent. of Acid of 1·20 sp. gr.	Specific Gravity.	Per cent. of HCL.	Per cent. of Acid of 1·20 sp. gr.
1·2000	40·777	100	1·328	26·913	66	1·0657	13·456	33
1·1982	40·369	99	1·308	26·505	65	1·0637	13·049	32
1·1964	39·961	98	1·1287	26·098	64	1·0617	12·641	31
1·1946	39·554	97	1·1267	25·690	63	1·0597	12·233	30
1·1928	39·146	96	1·1247	25·282	62	1·0577	11·825	29
1·1910	38·738	95	1·1226	24·847	61	1·0557	11·418	28
1·1893	38·330	94	1·1206	24·466	60	1·0537	11·010	27
1·1875	37·923	93	1·1185	24·058	59	1·0517	10·602	26
1·1857	37·516	92	1·1164	23·650	58	1·0497	10·194	25
1·1846	37·108	91	1·1143	23·242	57	1·0477	9·786	24
1·1822	36·700	90	1·1123	22·834	56	1·0457	9·379	23
1·1802	36·292	89	1·1102	22·426	55	1·0437	8·971	22
1·1782	35·884	88	1·1082	22·019	54	1·0417	8·563	21
1·1762	35·476	87	1·1061	21·611	53	1·0397	8·155	20
1·1741	35·068	86	1·1041	21·203	52	1·0377	7·747	19
1·1721	34·660	85	1·1020	20·796	51	1·0357	7·340	18
1·1701	34·252	84	1·1000	20·388	50	1·0337	6·932	17
1·1681	33·845	83	1·0980	19·980	49	1·0318	6·524	16
1·1661	33·437	82	1·0960	19·572	48	1·0298	6·116	15
1·1641	33·029	81	1·0939	19·165	47	1·0279	5·709	14
1·1620	32·621	80	1·0919	18·757	46	1·0259	5·301	13
1·1599	32·213	79	1·0899	18·349	45	1·0239	4·893	12
1·1578	31·805	78	1·0879	17·941	44	1·0220	4·486	11
1·1557	31·398	77	1·0859	17·534	43	1·0200	4·078	10
1·1536	30·990	76	1·0838	17·126	42	1·0180	3·670	9
1·1515	30·582	75	1·0818	16·718	41	1·0160	3·262	8
1·1494	30·174	74	1·0798	16·310	40	1·0140	2·854	7
1·1473	29·767	73	1·0778	15·902	39	1·0120	2·447	6
1·1452	29·359	72	1·0758	15·494	38	1·0100	2·039	5
1·1431	28·951	71	1·0738	15·087	37	1·0080	1·631	4
1·1410	28·544	70	1·0718	14·679	36	1·0060	1·224	3
1·1389	28·136	69	1·0697	14·271	35	1·0040	0·816	2
1·1369	27·728	68	1·0677	13·863	34	1·0020	0·408	1
1·1349	27·321	67						

Ure's Table showing the Percentages of HNO_3 corresponding to the Dilute Acid of Various Specific Gravities at 15° C.

HNO_3 per cent.	Specific Gravity. At 0°.	Specific Gravity. At 15°.	HNO_3 per cent.	Specific Gravity. At 0°.	Specific Gravity. At 15°.	HNO_3 per cent.	Specific Gravity. At 0°.	Specific Gravity. At 15°.
100·00	1·559	1·530	72·39	1·455	1·432	46·64	1·312	1·295
99·84	1·559	1·530	71·24	1·450	1·429	45·00	1·300	1·284
99·72	1·558	1·530	69·96	1·444	1·423	43·53	1·291	1·274
99·52	1·557	1·529	69·20	1·441	1·419	42·00	1·280	1·264
97·89	1·551	1·523	68·00	1·435	1·414	41·00	1·274	1·257
97·00	1·548	1·520	67·00	1·430	1·410	40·00	1·267	1·251
96·00	1·544	1·516	66·00	1·425	1·405	39·00	1·260	1·244
95·27	1·542	1·514	65·07	1·420	1·400	37·95	1·253	1·237
94·00	1·537	1·509	64·00	1·415	1·395	36·00	1·240	1·225
93·01	1·533	1·506	63·59	1·413	1·393	35·00	1·234	1·218
92·00	1·529	1·503	62·00	1·404	1·386	33·86	1·226	1·211
91·00	1·526	1·499	61·21	1·400	1·381	32·00	1·214	1·198
90·00	1·522	1·495	60·00	1·393	1·374	31·00	1·207	1·192
89·56	1·521	1·494	59·59	1·391	1·372	30·00	1·200	1·185
88·00	1·514	1·488	58·88	1·387	1·368	29·00	1·194	1·179
87·45	1·513	1·486	58·00	1·382	1·363	28·00	1·187	1·172
86·17	1·507	1·482	57·00	1·376	1·358	27·00	1·180	1·166
85·00	1·503	1·478	56·10	1·371	1·353	25·71	1·171	1·157
84·00	1·499	1·474	55·00	1·365	1·346	23·00	1·153	1·138
83·00	1·495	1·470	54·00	1·359	1·341	20·00	1·132	1·120
82·00	1·492	1·467	53·81	1·358	1·339	17·47	1·115	1·105
80·96	1·488	1·463	53·00	1·353	1·335	15·00	1·099	1·089
80·00	1·484	1·460	52·33	1·349	1·331	13·00	1·085	1·077
79·00	1·481	1·456	50·99	1·341	1·323	11·41	1·075	1·067
77·66	1·476	1·451	49·97	1·334	1·317	7·22	1·050	1·045
76·00	1·469	1·445	49·00	1·328	1·312	4·00	1·026	1·022
75·00	1·465	1·442	48·00	1·321	1·304	2·00	1·013	1·010
74·01	1·462	1·438	47·18	1·315	1·298	0·00	1·000	0·999
73·00	1·457	1·435						

TABLE SHOWING THE PERCENTAGE OF K_2O AND KHO IN SOLUTIONS OF CAUSTIC POTASH OF VARIOUS SPECIFIC GRAVITIES AT 15° C.*

Per cent. of K_2O.	Per cent. of KHO.	Specific Gravity.	Per cent. of K_2O.	Per cent. of KHO.	Specific Gravity.
·5658	0·674	1·0050	23·764	28·303	1·2648
1·697	2·021	1·0153	24·895	29·650	1·2805
2·829	3·369	1·0260	26·027	30·998	1·2966
3·961	4·717	1·0369	27·158	32·345	1·3131
5·002	5·957	1·0478	28·290	33·693	1·3300
6·224	7·412	1·0589	29·34	34·94	1·30
7·355	8·760	1·0703	30·74	36·61	1·32
8·487	10·108	1·0819	32·14	38·28	1·34
9·619	11·456	1·0938	33·46	39·85	1·36
10·750	12·803	1·1059	34·74	41·37	1·38
11·882	14·151	1·1182	35·99	42·86	1·40
13·013	15·498	1·1308	37·97	45·22	1·42
14·145	16·846	1·1437	40·17	47·84	1·44
15·277	18·195	1·1568	42·31	50·39	1·46
16·408	19·542	1·1702	44·40	52·88	1·48
17·540	20·890	1·1839	46·45	55·32	1·50
18·671	22·237	1·1979	48·46	57·71	1·52
19·803	23·585	1·2122	50·09	59·65	1·54
20·935	24·933	1·2268	51·58	61·43	1·56
21·500	25·606	1·2342	53·06	63·19	1·58
22·632	26·954	1·2493			

* Tünnermann and Richter.

TABLE SHOWING THE PERCENTAGE OF Na_2O IN SOLUTIONS OF CAUSTIC SODA OF VARIOUS SPECIFIC GRAVITIES AT 15° C.†

Per cent. of Na_2O.	Specific Gravity.	Per cent. of Na_2O.	Specific Gravity.	Per cent. of Na_2O.	Specific Gravity.
·302	1·0040	10·879	1·1630	21·154	1·3053
·604	1·0081	11·484	1·1734	21·758	1·3125
1·209	1·0163	12·088	1·1841	21·894	1·3143
1·813	1·0246	12·692	1·1948	22·363	1·3198
2·418	1·0330	13·297	1·2058	22·967	1·3273
3·022	1·0414	13·901	1·2178	23·572	1·3349
3·626	1·0500	14·506	1·2280	24·176	1·3426
4·231	1·0587	15·110	1·2392	24·780	1·3505
4·835	1·0675	15·714	1·2453	25·385	1·3586
5·440	1·0764	16·319	1·2515	25·989	1·3668
6·044	1·0855	16·923	1·2578	26·594	1·3751
6·648	1·0948	17·528	1·2642	27·200	1·3836
7·253	1·1042	18·132	1·2708	27·802	1·3923
7·857	1·1137	18·730	1·2775	28·407	1·4011
8·462	1·1233	19·341	1·2843	29·011	1·4101
9·066	1·1330	19·945	1·2912	29·616	1·4193
9·670	1·1428	20·550	1·2982	30·220	1·4285
10·275	1·1528				

† Tünnermann.

TABLE SHOWING THE PERCENTAGE OF NH_3 IN AQUEOUS SOLUTIONS OF THE GAS OF VARIOUS SPECIFIC GRAVITIES AT 14° C.*

Specific Gravity.	NH_3 per cent.	Specific Gravity.	NH_3 per cent.	Specific Gravity.	NH_3 per cent.
0·8844	36	0·9133	24	0·9520	12
0·8864	35	0·9162	23	0·9556	11
0·8885	34	0·9191	22	0·9593	10
0·8907	33	0·9221	21	0·9631	9
0·8929	32	0·9251	20	0·9670	8
0·8953	31	0·9283	19	0·9709	7
0·8976	30	0·9314	18	0·9749	6
0·9001	29	0·9347	17	0·9790	5
0·9026	28	0·9380	16	0·9031	4
0·9052	27	0·9414	15	0·9873	3
0·9078	26	0·9449	14	0·9915	2
0·9106	25	0·9484	13	0·9959	1

* Carius.

TABLE SHOWING SPECIFIC GRAVITY CORRESPONDING TO DEGREES, TWADDELL, BAUMÉ, AND BECK, FOR LIQUIDS HEAVIER THAN WATER.

Number of Degrees.	Corresponding Sp. Gr.			Number of Degrees.	Corresponding Sp. Gr.		
	Twaddell.	Baumé.	Beck.		Twaddell.	Baumé.	Beck.
0	1·000	1·000	1·000	21	1·105	1·166	1·1409
1	1·005	1·007	1·0059	22	1·110	1·176	1·1486
2	1·010	1·014	1·0119	23	1·115	1·185	1·1565
3	1·015	1·020	1·0180	24	1·120	1·195	1·1644
4	1·020	1·028	1·0241	25	1·125	1·205	1·1724
5	1·025	1·034	1·0303	26	1·130	1·215	1·1806
6	1·030	1·041	1·0366	27	1·135	1·225	1·1888
7	1·035	1·049	1·0429	28	1·140	1·235	1·1972
8	1·040	1·057	1·0494	29	1·145	1·245	1·2057
9	1·045	1·064	1·0559	30	1·150	1·256	1·2143
10	1·050	1·072	1·0625	32	1·160	1·278	1·2319
11	1·055	1·080	1·0692	34	1·170	1·300	1·2500
12	1·060	1·088	1·0759	36	1·180	1·324	1·2680
13	1·065	1·096	1·0828	38	1·190	1·349	1·2879
14	1·070	1·104	1·0897	40	1·200	1·375	1·3077
15	1·075	1·113	1·0968	45	1·225	1·442	1·3600
16	1·080	1·121	1·1039	50	1·250	1·515	1·4167
17	1·085	1·130	1·1111	55	1·275	1·596	1·4783
18	1·090	1·138	1·1184	60	1·300	1·690	1·5454
19	1·095	1·147	1·1258	65	1·325	1·793	1·6190
20	1·100	1·157	1·1333	70	1·350	1·909	1·7000

Boiling Point of Water under Different Pressures.

Pressure in Atmospheres.	Temperature. °F.	Temperature. °C.	Pressure in Atmospheres.	Temperature. °F.	Temperature. °C.
1	212·0	100·0	12	374·0	190·0
1·5	233·9	112·2	14	386·9	197·2
2	250·5	121·4	16	398·4	203·6
3	275·1	135·1	18	408·9	209·4
4	293·7	145·4	20	418·4	214·7
5	307·5	153·1	25	439·3	226·3
6	320·3	160·2	30	457·1	236·2
7	331·7	166·5	35	472·6	244·8
8	341·7	172·1	40	486·5	252·5
10	358·8	181·6	45	510·6	265·9

Temperature of Steam under Different Pressures.

Pressure per Square Inch.	Temperature. °F.	Temperature. °C.	Pressure per Square Inch.	Temperature. °F.	Temperature. °C.
Lbs.			Lbs.		
0·0	212·0	100·0	55·3	302·9	150·4
0·3	213·1	100·6	60·3	307·5	153·0
2·3	219·6	104·2	65·3	312·0	155·5
4·3	225·3	107·4	70·3	316·1	157·8
6·3	230·6	110·3	75·3	320·2	160·1
8·3	235·5	113·0	80·3	324·1	162·2
10·3	240·1	115·6	85·3	327·9	164·4
15·3	250·4	121·3	95·3	334·6	167·0
20·3	259·3	126·2	105·3	341·1	171·7
25·3	267·3	130·7	115·3	347·2	175·1
30·3	274·4	134·6	125·3	352·9	178·2
35·3	281·0	138·3	145·3	363·4	184·1
40·3	287·1	141·7	165·3	372·9	189·4
45·3	292·7	144·8	185·3	381·7	194·2
50·3	298·0	147·7	235·3	401·1	205·0

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